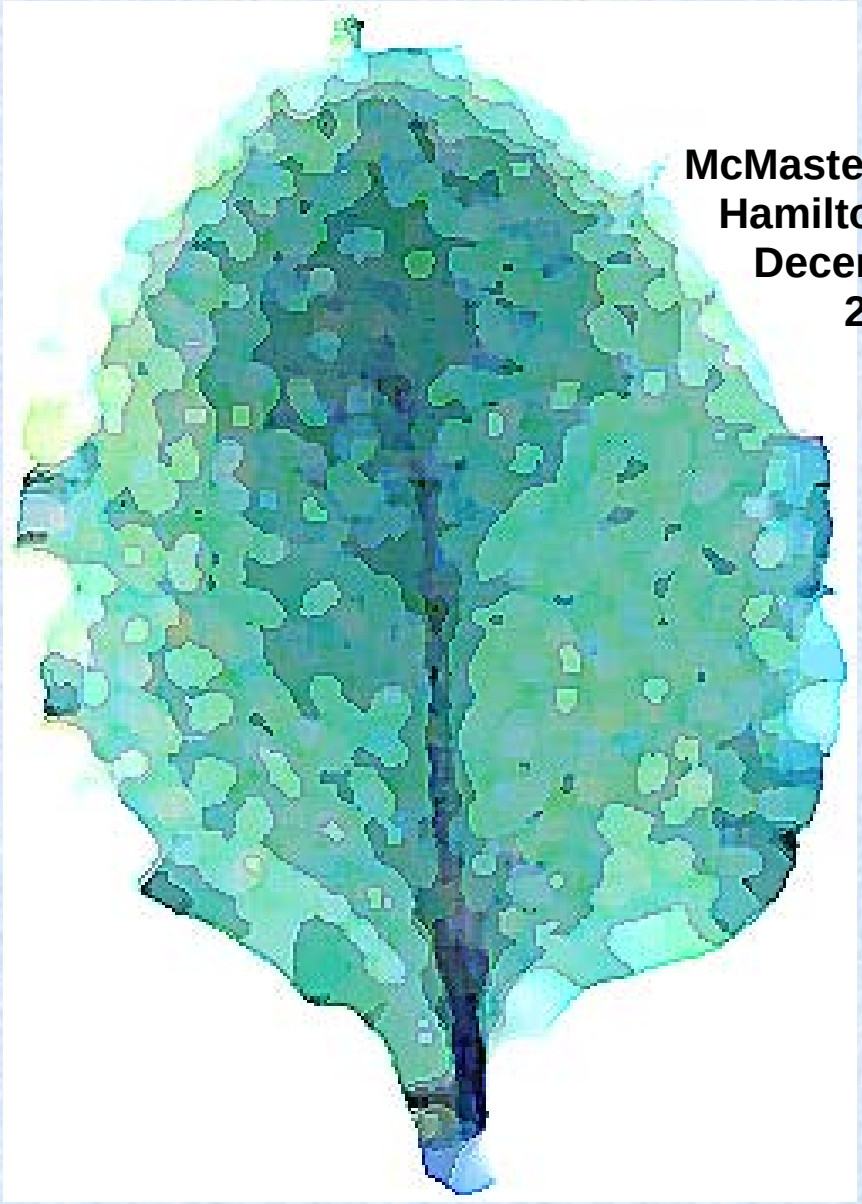
Proceedings of the joint meeting of

The Canadian Society of Plant Physiologists Eastern Regional Meeting & the 40th Plant Development Workshop



McMaster University
Hamilton Ontario
December 2nd
2006

Délibérations du congrès joint de la

Société Canadienne de Physiologie Végétale (Congrès régional de l'est) & le 40e Congrès de Développement Végétale

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Cover image: A GUS-stained Arabidopsis leaf creatively manipulated in Adobe Photoshop by Robin Cameron.

McMaster University welcomes you to the 2006 Eastern Regional Meeting of the Canadian Society of Plant Physiologists/Société Canadienne de Physiologie Végétale and the 40th Plant Development Workshop

We thank the following sponsors for their support

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CSPP/SCPV and PDW Program

December 1 and 2, 2006
Location: McMaster University, Michael DeGroote Centre of Leadership and Learning (MDCL)

December 1 (LS)

7:00 -10:00 pm, Life Sciences Bldg. Rm 213 … Meet and Greet for those in town

December 2 (MDCL)

8:00 - 8:50 am …….Registration and Coffee

8:00 - 9:00 am……..Poster Setup

8:50 - 9:00 am……. Opening Remarks

9:00 - 9:55 am……. 1st Keynote Speaker
Dan Klessig, Boyce Thompson Institute
"Salicylic acid, salicylic acid- binding proteins and plant immunity"

9:55 - 10:15 am……. Coffee Break

10:15 - 11:10 am……. 2nd Keynote Speaker
Sally Assmann, Pennsylvania State University
"Systems biology of hormone signaling in guard cells"

11:15 am - 12:30 pm……...Oral Presentations

12:00 - 2:00 pm……CSPP Executive Meeting

12:30 - 2:00 pm. ……. Lunch and Poster Presentations

2:00 - 2:55 pm……. 3rd Keynote Speaker
Jaideep Mathur, University of Guelph
"Early intracellular response profiling in higher plants"

3:00 - 5:00 pm…….…….Oral Presentations

5:00 - 5:30 pm……..Awards Committee Meeting

5:00 - 7:00 pm……..Wine & Cheese Reception, Awards

Session Schedule

Time	Session number	Location
Morning 9:15-12:30	I: Water, Ions and Mycorrhizae II: Proteins and Metabolism III: Abiotic stress	MDCL – 1110 MDCL – 1102 MDCL – 1105
Afternoon 3:00 - 5:00	IV: Anatomy and Development V: Signaling pathways VI: Respiration, Heavy metals, Phytoalexins and Pathogens	MDCL – 1110 MDCL – 1102 MDCL – 1105

Detailed Schedule

Time	Event	Location
Morning		
8:00 - 8:50	Registration and Coffee	MDCL hallway
8:00 - 9:00	Poster Setup	MDCL - South Hallway
8:50 - 9:00	Opening Remarks: Robin Cameron, Turlough Finan	MDCL – 1105
9:00 - 9:55	1st Keynote Speaker: **Dan Klessig** **Salicylic acid, salicylic acid-binding proteins and plant immunity**	MDCL – 1105
9:55 - 10:15	Coffee Break	MCDL hallway
10:15 - 11:10	2nd Keynote Speaker: **Sally Assmann** **Systems biology of hormone signaling in guard cells**	MDCL – 1105
	Oral Presentations	
11:15 - 12:30	**Session I: Water, Ions and Mycorrhizae** Chair: Ewa Cholewa	MDCL – 1110
11:15 - 11:30	**Changes in the permeability of soybean seed coats to water and PTS during development** Kosala M. Ranathunge and Carol A. Peterson	
11:30 - 11:45	**The effects of mechanical damage on water transport in leaves** Jennifer Janusz and Ewa Cholewa	
11:45 - 12:00	**Investigating the structure and function of *Iris germanica* roots** Chris J. Meyer, Ernst Steudle and Carol A. Peterson	
12:00 - 12:15	**Effect of exodermal development and epidermal death on ion uptake by onion roots** Ishari Waduwara and Carol A. Peterson	
12:15 - 12:30	**Analysis of natural and commercial inoculums on VAM infection in *Fragaria virginiana*** Jeff Taylor, Tyler Bintrim, Sara Mullendore, Carine Collin and Tia-Lynn Ashman	

11:15 - 12:30	**Session II: Proteins and Metabolism** Chair: Jessie Carviel	MDCL – 1102
11:15 - 11:30	**Identification of candidate tail-anchored membrane proteins in plant, yeast and mammalian proteomes: characterization of the localization and targeting signals of several novel Arabidopsis TA proteins** Priya K. Dhanoa, Meeta Mistry, Denil Wickrama, Xuemei Yang, Matthew P. Henderson Steven Primorac, Yeen Ting Hwang, Jon Lovell, David W. Andrews and Robert T. Mullen	
11:30 - 11:45	**Cloning and characterization of three Pathogenesis-Related proteins in European plums** Ashraf El-Kereamy, Jayasankar Subramanian and Deena Errampalli	
11:45 - 12:00	**Proteome analysis of soybean developing seed coats** M C Romero, D Brown and M Gijzen	
12:00 - 12:15	**Molecular mapping of genes involved in the phenylpropanoid pathway in bean (*Phaseolus vulgaris* L.)** Zeinab Yadegari and K. Peter Pauls	
12:15 - 12:30	**The role of debranching enzymes in starch metabolism** Thierry Delatte, Martin Umhang, Martine Trevisan and Samuel C. Zeeman	
11:15 - 12:30	**Session III: Abiotic stress** Chair: David Guevara	MDCL – 1105
11:15 - 11:30	**Increased temperature during autumn conditions inhibits photosynthetic carbon gain in seedlings of *Pinus banksiana*** Florian Busch, Norman P.A. Hüner and Ingo Ensminger	
11:30 - 11:45	**The role of excitation pressure on the extent of variegation in the *immutans* mutant of *Arabidopsis thaliana*** Dominic Rosso, Diego Saccon, Shelly Wang, Steven R. Rodermel, Denis P. Maxwell and Norman P.A. Huner	
11:45 - 12:00	**High growth temperatures reduce photosynthesis, respiration and growth in black spruce** Danielle A. Way and Rowan F. Sage	
12:00 - 12:15	**Increase in specific proteins associated with the desiccation of tolerant roots of a horticultural dicot, *Ranunculus asiaticus.*** Yujie Gong and J. Derek Bewley	
12.15 - 12.30	**Metabolic responses of *Thellungiella salsuginea* during acclimation to osmotic stress imposed under field or growth chamber conditions** David Guevara, Brian McCarry, Peter Summers and Elizabeth Weretilnyk	

Afternoon		
12:00 - 2:00	CSPP Executive Meeting	MDCL - 1116
12:30 - 2:00	Lunch	MDCL hallway 1009, 1010
	Poster Presentations	MDCL - South Hallway
2:00 - 2:55	3rd Keynote Speaker: **Jaideep Mathur** **Early intracellular response profiling in higher plants**	MDCL – 1105
	Oral Presentations	
3:00 - 5:00	**Session IV: Anatomy & Development** Chair: Usher Posluszny	MDCL – 1110
3:00 - 3:15	***GAMYB* gene expression and seed germination in tomato** Xuemei Gong and J. Derek Bewley	
3:15 - 3:30	**Developmental morphology of several vine members of the Cucurbitaceae** Tim Zitnak and Usher Posluszny	
3:30 - 3:45	**Analysis of *Eriophorum vaginatum* corms, sclereid clusters, and adjacent substrate for 9 industrial metals related to mining in Sudbury, ON** Sarah Bogart, Ewa Cholewa and Graeme Spires	
3:45 - 4:00	**The role of abscission during leaflet separation in *Chamaedorea elegans* (Arecaceae)** Julia Nowak, Nancy G. Dengler and Usher Posluszny	
4:00 - 4:15	**Break**	
4:15 - 4:30	**Ecotype screen for sequence and splicing variation at the MADS-AFFECTING FLOWERING LOCUS** Sarah Rosloski and Vojislava Grbic	
4:30 - 4:45	**Tissue specific accumulation of cyanogens and dynamics of cyanide levels in peach, plum and ornamental *Prunus* spp.** Anna Kalinina, Brian McGarvey, Ralph Chapman and Daniel C.W. Brown	
4:45 - 5:00	**Identification and location of a candidate gene controlling peach fruit fuzz** Ali Taheri, Jayasankar Subramanian, Peter Pauls, Manish Raizada and John Cline	

3:00 - 5:00	**Session V: Signaling pathways** Chair: Fadi Al-daoud	MDCL – 1102
3:00 - 3:15	**Characterisation of the PERK family of receptor kinases: an emerging cytoskeleton connection** T.V. Humphrey, K.E. Haasen, M.G. Aldea and D.R. Goring	
3:15 - 3:30	**A mutation in *LINES*, which encodes a RING-type ubiquitin ligase, disrupts *Arabidopsis* adaptability to nitrogen limitation and alters the responsive transcriptome** Mingsheng Peng, Carol Hannam, Honglan Gu, Yong-Mei Bi and Steven J. Rothstein	
3:30 - 3:45	**Islands of co-expressed neighbouring genes in *Arabidopsis thaliana* suggest global regulation of chromatin structure** Shuhua Zhan, J. Horrocks and L.N. Lukens	
3:45 - 4:00	**The Arabidopsis TGA2 repressor binds the co-activator NPR1 to form an SA-regulated enhanceosome** Amanda Rochon, Patrick Boyle, Tracy Wignes, Pierre R. Fobert and Charles Despres	
4:00 - 4:15	**Break**	
4:15 - 4:30	**The NPR1 BTB/POZ domain is required for both TGA2-dependent NPR1 coactivator function and PR-1 gene induction** Amanda Rochon, Patrick Boyle, Tracy Wignes, Pierre R. Fobert and Charles Després	
4:30 - 4:45	**The chimeric cyclic nucleotide-gated ion channel AtCNGC11/12 induces programmed cell death** William Urquhart, Arunika N. Gunawardena, Wolfgang Moeder, Rashid Ali, Gerald Berkowitz and Keiko Yoshioka	
4:45 - 5:00	**Identification of a functionally essential amino acid for the cyclic nucleotide gated ion channel ATCNGC11/12** Joyce Baxter, Wolfgang Moeder, Maggie Wang, William Urquhart, Dinesh Christendat and Keiko Yoshioka	
3:00 - 5:00	**Session VI: Respiration, Heavy metals, Phytoalexins and Pathogens** Chair: Sheila Macfie	MDCL – 1105
3:00 - 3:15	**Mesocarp localization of a bifunctional resveratrol/hydroxycinnamic acid glucosyltransferase of Concord grape (*Vitis labrusca*)** Dawn Hall and Vincenzo De Luca	
3:15 - 3:30	**An *Arabidopsis* glyoxylate/pyruvate-dependent GABA transaminase** Shawn M. Clark and Barry J. Shelp	

3:30 - 3:45	**Functional analysis of an Arabidopsis succinic semialdehyde reductase** Wendy L Allan, Gordon J. Hoover and Barry J. Shelp	
3:45 - 4:00	**Dynamics of arbuscular mycorrhizal symbiosis in heavy metal phytoremediation** Patrick Audet and Christiane Charest	
4:00 - 4:15	**Break**	
4:15 - 4:30	**Taxonomic distribution of alternative oxidase and plastoquinol terminal oxidase in all kingdoms of life** Allison E. McDonald and Greg C. Vanlerberghe	
4:30 - 4:45	**Analysis of RNA silencing suppressor activity by TGBp1 of *Pepino Mosaic Virus*** Fariba Shahmir and Annette Nassuth	
4:45 - 5:00	**Molecular approaches toward improving *Fusarium graminearum* resistance in corn** Yarmilla Reinprecht, Shun-Yan Luk, Cristopher J. Martin and K. Peter Pauls	
5:00 - 5:30	Awards Committee Meeting	MDCL - 1116
5:00 - 7:00	Wine & Cheese Reception, Awards	MDCL South Hallway

Poster Presentations

P1: The role of *IAP1* in age-related resistance
Jessie Carviel and Robin K. Cameron

P2: Assessment of the metabolic activity of chamber-grown *Fragaria virginiana* during flowering
Sarah Dobson, Carine Collin, Tia-Lynn Ashman and Jeff H. Taylor

P3: Identification and characterization of *cis*-acting elements in *CHS7* and *CHS8* gene promoters in *Glycine max*
Jinxin Yi and Sangeeta Dhaubhadel

P4: Harvesting *Catharanthus* leaf epidermis proteins and mRNA by Carborundum abrasion technique: Molecular cloning and functional characterization of Epidermis specific 16-hytroxytabersonine-16-*O*-methyltransferase (16-OMT)
Dylan Levac, Jun Murata and Vincenzo De Luca

P5: *Catharanthus roseus* somatic embryo development and monoterpenoid indole alkaloid biosynthesis
Jun Murata, Jian-Xin, Chen and VIncenzo De Luca

P6: Natural genetic variation for water stress responses in *Zea mays* roots
Tina Wambach and Lewis Lukens

P7: Transgressive segregation for herbivory resistance in *Brassica napus*
Xiaoguang Wu, Laima Kott and Lewis Lukens

P8: Testing the usefulness of a cDNA microarray as a screen for high-isoflavone soybean lines
Scott Fife, I. Rajcan and K.P. Pauls

P9: Characterization of the nodulation phenotype of E151, a pleiotropic pea (*Pisum sativum L.*) mutant
Michael Chlup and Frédérique Guinel

P10: Identification of common bacterial blight resistance genes in *Phaseolus vulgaris*
Perry G.E., Reinprecht Y and Pauls K.P.

P11: The effect of storage protein deficiency on protein composition in mature seeds of common bean
Meghan Taylor, Frédéric Marsolais and Ralph Chapman

P12: Cuticular cracks in soybean seed coats: development and effect on permeability
Elizabeth Bernath and Carol A. Peterson

P13: The role of two *NAC*-like transcription factors during Age-Related Resistance in Arabidopsis
Fadi Al-daoud, A. Mohammad, M Neumann, J. Carviel and Robin K. Cameron

P14: Systems biology of *Pseudomonas syringae* virulence
Karl Schreiber and Darrell Desveaux

P15: Methylation in the chloroplast DNA of flax (*Linum usitatissimum*)
Michelle M. De Decker, Matthew D. Smith and Mary Ann Fieldes

P16: Kinetic analysis of a recombinant *Arabidopisis* succinate semialdehyde/glyoxylate reductase
Barry J. Shelp, Gordon J. Hoover, A. Rod Merrill and Shawn M. Clark

P17: Transformation of *Acinetobacter* sp. with the *cp4 epsps* gene
Rachel R. Campbell, Robert H. Gulden, David J. Levy-Booth, Miranda M. Hart, Jeff R. Powell, Kari E. Dunfield, Jack T. Trevors, John P. Klironomos, Clarence J. Swanton and K. Peter Pauls

P18: AtFIN3: A host target of the *Pseudomonas syringae* Type III effector HopF
Mike Wilton, Yaqi Hu and Darrell Desveaux

P19: Suppression and induction among six members of the defensin gene family in *Nicotiana benthamiana* during compatible and incompatible plant-pathogen interactions
Bahman Bahramnejad, Larry Erickson and Paul Goodwin

P20: Expression of the porcine β-defensin-1 (*pbd-1*) gene in plants
Chuthamat Atnaseo, Larry Erickson and Paul Goodwin

P21: Identification of host targets of *Pseudomonas syringae* HopZ cysteine proteases
Jennifer D. Lewis, David S. Guttman and Darrell Desveaux

P22: Protein-protein interactions between maize starch synthases and branching enzymes expressed in *E. coli*
Fushan Liu, Ian J.Tetlow and Michael J. Emes

P23: Dual localization of diacylglycerol acyltransferase 1, an enzyme involved in the biosynthesis of triacylglycerol, to ER and chloroplasts
Amy McNaughton, Marianne Hopkins, Matthew D. Smith and John E. Thompson

P24: Expression of therapeutic proteins in tobacco BY-2 cell suspension culture
Adil Ahmad, Angelo Kaldis and Rima Menassa

P25: The *Arabidopsis thaliana* 36 kDa peroxisomal membrane protein contains two distinct molecular targeting signals
G. S. T. Smith, U. A. Schumann and R. T. Mullen

P26: Using two putative *AROGENATE DEHYDRATASEs* (*ADT*s) from *Arabidopsis* to complement the *pha2* phenotype in yeast
Oliver R.A. Corea, Mark A. Bernards and Susanne E. Kohalmi

P27: Analysis of *AROGENATE DEHYDRATASE* (*ADT*) expression patterns in *Arabidopsis thaliana* using RT-PCR
Rebecca L. Hood and Susanne E. Kohalmi.

P28: Transient expression of *Arabidopsis* ADT1 and ADT3 as GFP fusions in onion epithelial cells
Gobi Thillainadesan and Susanne E. Kohalmi

P29: Is there a role for ESCRT in the formation of multivesicular peroxisomes during tombusvirus infection?
Alex S.M. Howard, Andrew W. McCartney, C. Robb Flynn, Graham S.T. Smith and Robert T. Mullen

P30: "Caterpillar-specific" transcriptional responses in the model legume, *Medicago truncatula*
Shireef A. Darwish and Jacqueline C. Bede

P31: Characterization of transgenic potato roots transformed to underexpress cytosolic triose phosphate isomerase
Sonia Dorion, Audrey Clendenning, Julie Jeukens, Andrea Haner, David R. Law and Jean Rivoal

P32: Cloning, purification and characterization of a cytosolic peroxiredoxin from *Solanum chacoense*
Émilie Maheux, Sonia Dorion, Daniel P. Matton and Jean Rivoal

P33: Partial purification of the most abundant hexokinase isoform from potato (*Solanum tuberosum*) tubers
Marie-Claude Moisan and Jean Rivoal

P34: Failed root growth in common bean (*Phaseolus vulgaris* L.) dwarf plants studied under different temperature regimes, externally supplied sucrose and $^{14}CO_2$
Muhammad J. Iqbal, Evangelos D. Leonardos and Bernard Grodzinski

P35: Investigating the role of the Actin cytoskeleton in maintaining cell-cell contact during plant development
Sujitha Muthuswamy, Ashleigh Downing, Neeta Mathur, John Greenwood and Jaideep Mathur

P36: Dissecting subcellular responses to ROS using simultaneous multicolour live imaging in *Arabidopsis thaliana*
Alison Sinclair and Jaideep Mathur

P37: Stability of starch biosynthetic enzyme complexes in maize endosperm
Sanjeena Subedi, Amina Makhmoudova, Ian J.Tetlow and Michael J. Emes

P38: Grape *CBF* genes increase stress tolerance and change phenotype in *Arabidopsis*
Mahbuba Siddiqua and Annette Nassuth

P39: Impact of sex, reproductive status and soil conditions on the mycorrhizal status of wild strawberry plants (*Fragaria virginiana*).
Sara Mullendore, Ty Bintrim, Carine Collin, Tia-Lynn Ashman and Jeff H. Taylor

P40: Response of Thellungiella to water deficits
Jeff Dedrick and Elizabeth Weretilnyk

P41: Involvement of the *Solanum chacoense* MAPKKK ScFRK1 in embryo sac development
Edith Lafleur, Christelle Kapfer, Madoka Gray-Mitsumune and Daniel P. Matton

P42: Tiller senescence level determines the appearance of later leaves in rice
Raja Khanal and Folkard Asch.

P43: Do apoplastic barrier bypasses exist in roots?
Kyle Bender and Ewa Cholewa

P44: The C-terminal tail sequences of ER and mitochondrial cytochrome b_5 convey their organelle-specific targeting in plant cells – conservation of the targeting mechanism for mitochondrial tail-anchored proteins
Yeen Ting Hwang, Matthew Henderson, Preetinder K. Dhanoa, David W. Andrews, John M. Dyer and Robert T. Mullen

P45: The distribution of two major iridoids in different organs of *Antirrhinum majus* L. at selected stages of development.
Clifford W. Beninger, Renée R. Cloutier, Mario A. Monteiro and Bernard Grodzinski

P46: Two Arabidopsis plant U-box (AtPUB) E3 ubiquitin ligases may function as potential regulators of plant signalling pathways during abiotic stress
Donna Yee, Nicholas Provart and Daphne R. Goring

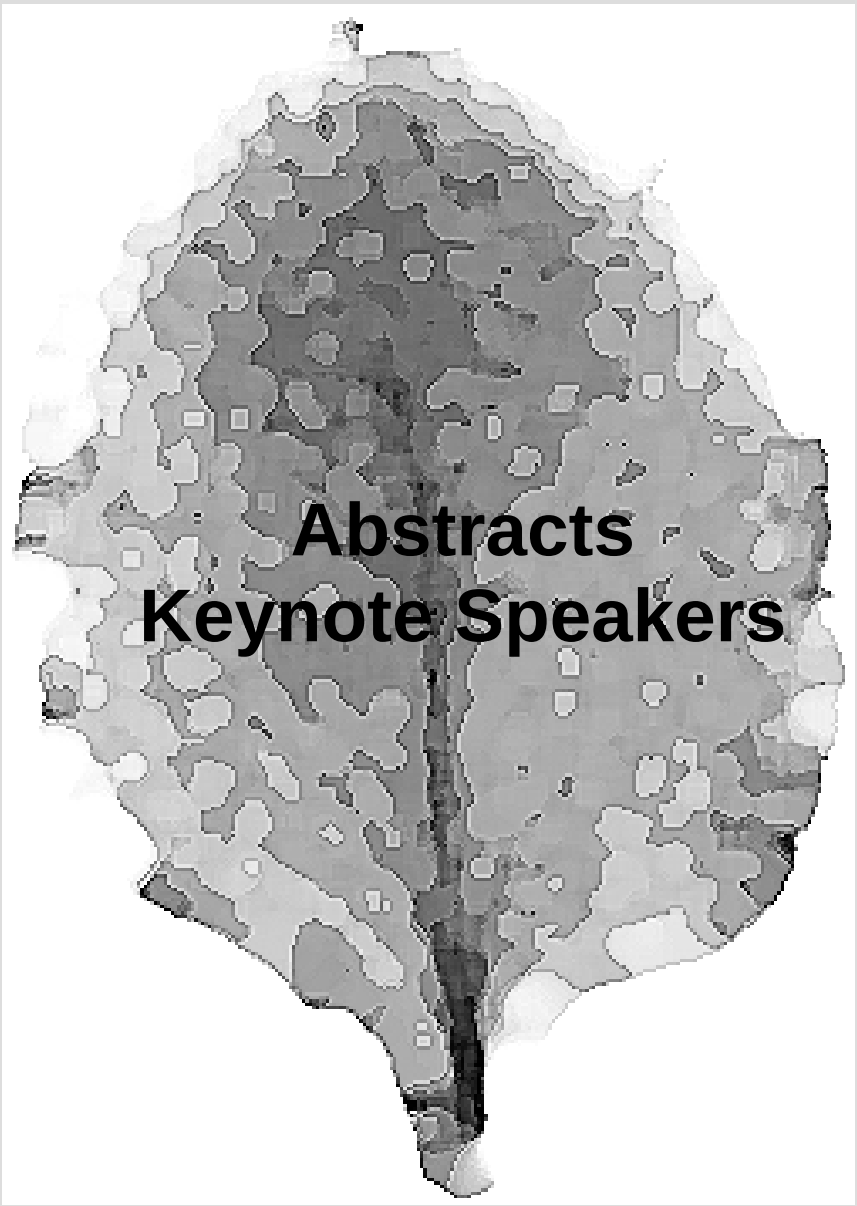
P47: Dark septate endophytes: isolation, identification and characterization toward their potential use as biological control agents against plant pathogens.
Richard Bourgault, Toshihiro Kamiya, Kazutoshi Nakamura and Yukari Kuga

P48: Growth temperature regulates colony formation in the psychrophilic and mesophilic strains of *Chlamydomonas raudensis*.
Beth Szyszka, Tessa Pocock, Ognjen Visnjevac and Norm Hüner

P49: Gene flow from *Sorghum bicolor* to its weedy relatives and its consequences
Titus Magomere, François Tardif and K. Peter Pauls

P50: Effects of cadmium on stomatal size and oxygen evolution
Renkang Zhu, Sheila M. Macfie and Zhifeng Ding

P51: Phosphorylation of PS II core proteins in *chlorina F2* barley mutant (*Hordeum vulgare* L. cv .Dornaria, Chlorinaf2-^{2800}) M. Krol, A.Ivanov, A.Mattoo, I. Booij-James, D. Rosso, N.P.A. Huner



Abstracts
Keynote Speakers

9:00 – 9:55 am, MDCL-1105

Salicylic acid, salicylic acid-binding proteins and plant immunity

Dr. Dan F. Klessig
Boyce Thompson Institute for Plant Research, Ithaca, New York, USA

Salicylic acid (SA) is a key plant hormone for defense against pathogens. To help understand how the SA signal is transmitted and SA's mechanisms of action, we have identified several SA-binding proteins (SABPs) in tobacco. These include catalase and ascorbate peroxidase, the two major H_2O_2-scavenging enzymes (Chen et al., Science 1993, 262:1883; Durner and Klessig, PNAS 1995, 92:11312). Their inhibition by SA may contribute to the oxidative burst, which accompanies infection by avirulent pathogens. A third protein in this group is SABP2. It is a methyl salicylate (MeSA) esterase that has high affinity for SA. The 3-D structure of SABP2, alone or in complex with SA, has been determined, confirming that it is a member of the α/β hydrolase superfamily. SA binds in the active site of SABP2, thereby inhibiting its esterase activity. Silencing of *SABP2* expression suppresses local resistance TMV and blocks the development of systemic acquired resistance (SAR) (Kumar and Klessig PNAS 2003, 100:16101; Forouhar et al. PNAS 2005, 102:1773). Our recent grafting and molecular genetic studies have extended theses findings. We found that SABP2's MeSA esterase activity is required in the systemic tissue to respond to the SAR signal, but is not needed in the primary infected tissue to generate this signal. Expression of mutant SABP2 displaying unregulated MeSA esterase activity compromises SAR if expressed in the primary infected tissue, presumably because this does not allow buildup of MeSA. MeSA levels increase in primary infected leaves, phloem exudates from these leaves and systemic leaves of control plants but not in these tissues of transgenic tobacco expressing the unregulated SABP2. Together these results argue that MeSA is the long-sought, phloem-mobile signal for SAR and that SABP2's role is to convert biologically inactive MeSA to active SA in the systemic tissue.

Notes

10:15 – 11:10 am, MDCL 1105

Systems biology of hormone signaling in guard cells

Dr. Sally Assmann
Department of Biology, Pennsylvania State University, USA

Heterotrimeric G-proteins, comprised of Gα, Gβ, and Gγ subunits, are ubiquitous and important signaling elements in eukaryotes, and ion channels are common downstream targets. The genome of the model plant species, *Arabidopsis thaliana,* contains one prototypical G-protein α subunit gene, *GPA1*. We are investigating the roles of heterotrimeric G proteins in abscisic acid (ABA) regulation of guard cell function. Pairs of guard cells border and regulate stomatal pores in the leaf epidermis, through which carbon dioxide uptake and water loss occur. Guard cells regulate stomatal apertures through dynamic changes in guard cell volume, mediated by changes in ion and water flux across the guard cell membrane. In wild-type guard cells, inhibition of inwardly rectifying K^+ channels and activation of Ca^{2+}-permeable channels and anion channels by abscisic acid (ABA) plays a crucial role in the inhibition of stomatal opening and promotion of stomatal closure in response to drought. We are investigating the ABA-regulation of these channels in *gpa1* mutants. G-proteins represent just one aspect of ABA control of guard cell function: ABA regulates stomatal apertures via an intricate intracellular network involving more than 40 known components. Methods developed by systems biologists can be useful tools to formalize such interactions and evaluate their relative importance. We have applied methods of network analysis to codify current knowledge concerning ABA promotion of stomatal closure.

Assmann, S.M. 2005. G proteins go green: A plant G protein signaling FAQsheet. **Science.** 310: 71-73.

Perfus-Barbeoch L, Jones AM, Assmann SM. 2004. Plant heterotrimeric G protein function: insights from Arabidopsis and rice mutants. **Curr Opin Plant Biol.** 7: 719-731.

Notes

2:00 – 2:55 pm, MDCL 1105

Early intracellular response profiling of plants

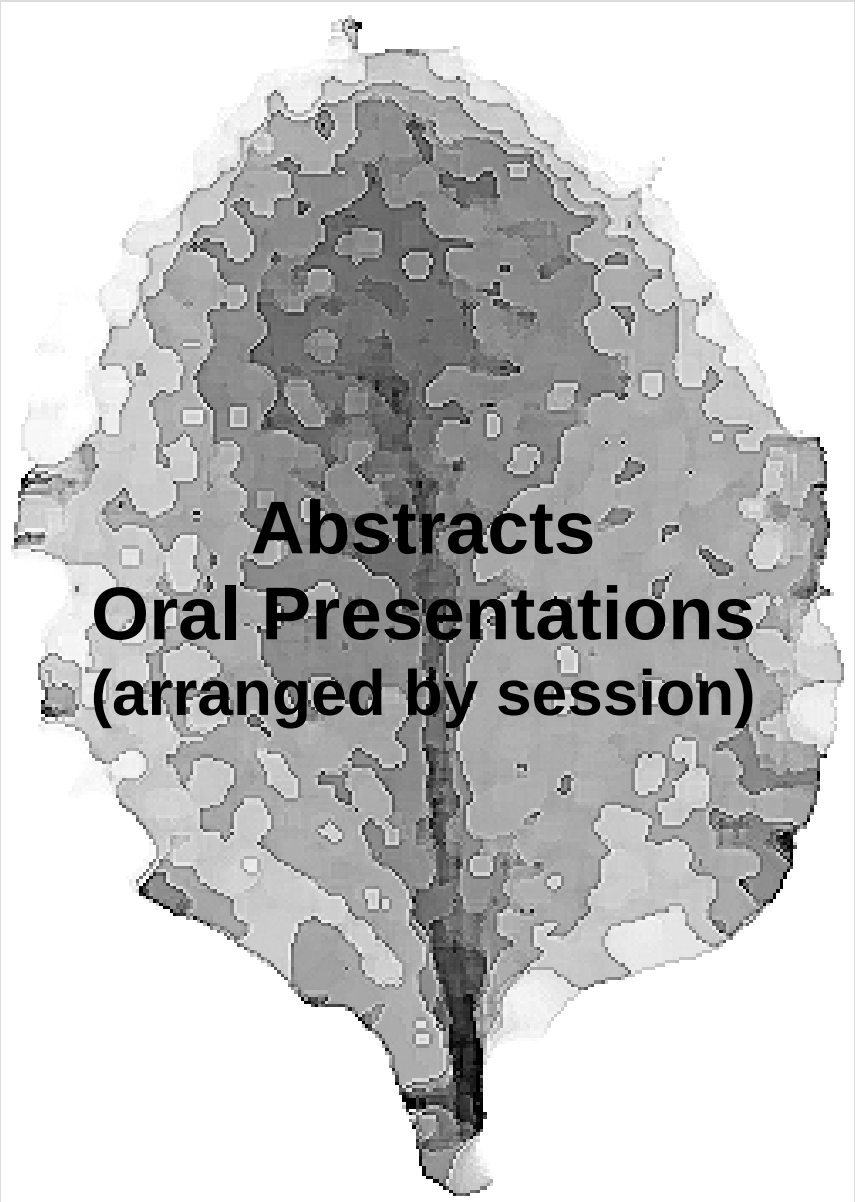
Dr. Jaideep Mathur
Department of Molecular & Cellular Biology, University of Guelph

The phenomenal progress in organismal genomics and proteomics seen in recent years has generated a large volume of data and defined a new challenge for the modern biologist; that of putting the isolated genes and their products back into their cellular / organismal context. Clearly our comprehension of an organism and its diverse responses to environmental stimuli will increase along with our ability to observe, document and dissect the laws that govern sub-cellular events and interactions. An elegant way of achieving this goal is to utilize fluorescent protein aided live-imaging technology for looking inside cells.

With the above backdrop in mind I have initiated a long-term project on "**E**arly **I**ntracellular **R**esponse **P**rofiling of **P**lants." <http://www.uoguelph.ca/~jmathur/Research/EIRP.html>. EIRPP is an integrative approach that capitalizes on annotated gene sequences available for different model plants <http://plantgenomics.tigr.org/>, utilizes publicly available mutant collections <http://www.biosci.ohio-state.edu/~plantbio/Facilities/abrc/abrchome.htm>, and employs a large number of transgenic lines carrying fluorescent proteins targeted to nearly all major 'text-book' organelles in a plant cell (Dhanoa et al. 2006). Our lab's present focus under the EIRPP program is on two areas: 1) Organelle behavior and interactions in response to oxidative stress; 2) Membrane-Cytoskeleton interactions affecting cell boundary extension in plants.
The advances in plant biology leading to the EIRPP approach along with our recent findings on sub-cellular dynamics and their implications will be presented.

Reference: Dhanoa PK, Sinclair A, Mullen RT, and Mathur J. Illuminating sub-cellular structures and dynamics in plants: A fluorescent protein toolbox. Can. J. Bot. 84(4): 515-522. 2006.

Notes



Abstracts
Oral Presentations
(arranged by session)

Session I: Water, Ions and Mycorrhizae
11:15 – 12:30 am, Rm: MDCL – 1110

1:Changes in the permeability of soybean seed coats to water and PTS during development

Kosala M. Ranathunge and Carol A. Peterson
Department of Biology, University of Waterloo
email: Kosala_Ranathunge@yahoo.co.uk

As with many legumes, soybean (*Glycine max* [L.] Merr.) plants produce some stone (impermeable) seeds that do not take up water for long periods of time. In contrast, permeable seeds readily imbibe water through cuticular cracks on the dorsal side of the seed coat. Previous work indicated that the outermost cuticle provides the greatest resistance to water passage through the seed coat. How does this system develop? In the present study, water movement through the seed coats of three cultivars was measured during seed development, categorized as early (E1 and E2), mid (M1 and M2), late (L1, L2a and L2b) and drying (D1, D2 and D3) stages. Water transport from intact seeds was measured by weight loss under conditions of constant temperature and humidity. Similar seeds were soaked for four hours in 0.1% PTS (an apoplastic tracer dye) to detect sites of penetration. In all cultivars, the permeability of the seed coat to water and PTS declined with seed development until the L2a stage. Cracks in the seed coat cuticle of permeable cultivars appeared at the L2b stage, while no cracks were observed in impermeable seeds. Crack development dramatically increased the water permeability of permeable seed coats compared to that of impermeable ones. Stone seed coats made a tight barrier for water and PTS movement during the drying stages. The seed hilum and micropyle area of all cultivars were leaky for water and PTS up to the D2 stage, but were sealed at the end of the drying period (D3).

2: The effects of mechanical damage on water transport in leaves

Jennifer Janusz and Ewa Cholewa
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Hierarchical venation within leaves allows for large amounts of water distribution and could allow for alternate pathways if the midrib becomes injured. Recent studies have suggested the midrib, although useful in water transport, may not be crucial to net leaf water dispersion (Sack *et al*. Whole Plant and Ecophysiol. 2004, 134:1824 ; Zwieniecki *et al*. Plant, Cell and Environ 2002, 25: 1445). The purpose of this study is to determine how leaves compensate when this major water pathway is lost. Three groups *Codiaeum variegatum* and *Musa sapientum L.* leaves were used in this study: One control group and two experimental groups whose midribs were cut in different locations. All leaves were placed in a 1% Safranin O solution and after forty-eight hours were observed for prevalent water pathways.

All experimentally injured leaves displayed different water distribution patterns from the control groups. One hundred percent of minor veins sampled within the lower portion of injured group leaves showed dye present in comparison to control groups which showed 0% to 50% within similarly sampled areas. These injured leaves did not rely on the midrib and water was transported through secondary, tertiary and in some cases, quaternary veins, bypassing the site of injury. Experimentally injured leaves also continued to transport large amounts of water despite the midrib being damaged and maintained normal leaf functions (continuing to function normally a year after damaged occurred within some leaves). These results suggest the midrib may be more of a convenient structure for mechanical support due to heavily lignified tracheary elements than a compulsory part of water distribution.

3: Investigating the structure and function of *Iris germanica* roots

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Roots of *Iris germanica* form a multi-layered exodermis in which the Casparian bands extend throughout the anticlinal and tangential cell walls to form a continuous band around the circumference (i.e., a continuous Casparian band, CCb). These cells also have suberin lamellae, which are well known as being hydrophobic. The development of these wall-modifying structures was influenced by growth conditions. In soil-grown roots, the structures formed very close to the root tip. However, in hydro- and aeroponic conditions, development was restricted. CCb formation was induced by introducing an 'air gap' in the hydroponic tank. Water and solute permeabilities of this special exodermis were measured using both a root pressure probe (RPP) and pressure chamber (RPC). The water permeability of roots with a CCb was $3.4 \pm 1.9 \times 10^{-7}$ m s^{-1} MPa^{-1} when using the RPP but was lower when measured with the RPC ($0.21 \pm 0.09 \times 10^{-7}$ m s^{-1} MPa^{-1}). These permeabilities increased, but variably, after the CCb was punctured ($7.6 \pm 5.4 \times 10^{-7}$ or $1.7 \pm 1.7 \times 10^{-7}$ m s^{-1} MPa^{-1}, RPP or RPC, respectively). Roots with an intact CCb completely blocked NaCl transport and were highly restrictive to ethanol passage ($0.075 \pm 0.18 \times 10^{-9}$ m s^{-1}). When the CCb was punctured, the root became permeable to NaCl ($9.9 \pm 6.1 \times 10^{-9}$ m s^{-1}) and slightly more permeable to ethanol ($1.4 \pm 2.7 \times 10^{-9}$ m s^{-1}). This was the first time that the permeability of a multi-layered exodermis has been measured quantitatively. In nature, *I. germanica*'s CCb would greatly limit solute loss while still being slightly permeable to water; the majority of water uptake would be close to the root tip.

4: Effect of exodermal development and epidermal death on ion uptake by onion roots

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During drought the exodermis matures close to the root tip and the epidermis dies, reducing the surface area of the plasma membrane available for ion uptake. Previously, exodermal maturation was predicted to reduce ion uptake but this idea was not tested experimentally. Do exodermal maturation and epidermal death reduce ion uptake in proportion to the loss of absorptive plasma membrane surface area? To answer this question, three anatomically distinct segments were isolated along the lengths of onion (*Allium cepa* L cv. Wolf) adventitious roots. In order of age, these areas were Immature Exodermis Live Epidermis (IEXLEP), Mature Exodermis Live Epidermis (MEXLEP), and Mature Exodermis Dead Epidermis (MEXDEP). The segments were treated with either radioactive phosphate or sulphate for 17 h prior to quantification of the ions in various compartments (surface, cell wall, cytoplasm, 'vacuole', and 'bound') using a compartmental elution technique. Quantities of both ions in the walls confirm that the exodermis is a barrier to free diffusion into the root (i.e., higher quantities of ions were present in the IEXLEP zone compared with mature exodermal zones). Cumulative quantities of ions in the cytoplasm, 'vacuole', and 'bound' (when present) compartments indicated that development of the exodermis reduced the quantities of sulphate but not phosphate that had moved across the plasma membranes. On the other hand, death of the epidermis reduced the absorbed quantities of both ions. The uptake of both ions was greater than expected considering the reductions in absorptive plasma membrane surface areas caused by exodermal maturation and epidermal death. Therefore, roots are capable of compensating for these events, and retain the capacity for considerable ion uptake.

5: Analysis of natural and commercial inoculums on VAM infection in *Fragaria virginiana.*

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Vesicular arbuscular mycorrhiza (VAM) are a mycorrhizal morphotype common to herbaceous plants. Generally, it is accepted that VAM inoculation is beneficial to plant growth, and this has lead to intensive assessment of their impact in agriculture or gardening practices. The media attention has lead to the formation of companies producing mycorrhizal inoculums. The benefit of this is questionable, given the prevalence of mycorrhizal spores in unadulterated soil. In the current study, the abilities of natural and commercial VAM inoculums to produce VAM associations in *Fragaria virginiana* (strawberry) were compared. Natural VAM inoculations were obtained from soil sample taken from Pymatuning State Park (Pennsylvania), while commercial VAM inoculations were procured from Plant Health Care, Inc (Pittsburgh, PA). Inoculations were applied to *F. virginiana* plants treated with captan to render them non-mycorrhizal. Using the presence of vesicles (made visible by Trypan blue staining) to indicate mycorrhizal prevalence, it was noted that the commercial inoculum generated VAM associations both more rapidly and to greater density than did any of the natural inoculums. Additionally, increasing the density of the commercial inoculum did not significantly impact the rate or ultimate densities at which VAM were formed in *F. virginiana*. The relevance of these findings to more typical settings and the potential impacts on plant growth will be discussed.

Notes

Session II: Proteins and Metabolism
11:15 – 12:30 am, Rm: MDCL – 1102

1: Identification of candidate tail-anchored membrane proteins in plant, yeast and mammalian proteomes: characterization of the localization and targeting signals of several novel Arabidopsis TA proteins

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Tail-anchored (TA) integral membrane proteins are found in almost all cellular membranes and are defined by the presence of an N-terminal cytosolic domain that represents the majority of the protein, a single transmembrane domain located near their C terminus, and a short C-terminal sequence that protrudes into the organelle lumen. While the biochemical functions and intracellular trafficking of some TA proteins have been well characterized, our understanding of TA protein biogenesis in general is limited because only a relatively few TA proteins have been identified in evolutionarily diverse organisms. In this study, we employed a newly-developed computer algorithm named TAMP (Tail-Anchored Membrane Proteins) to identify 508, 100 and 378 candidate TA proteins in the Arabidopsis, *Saccharomyces cerevisiae* and human proteomes, respectively. While many of these putative TA proteins have been functionally annotated using Gene Ontology, ~ 45% of the proteins identified in each proteome are of unknown function. Preliminary results obtained from localization experiments revealed that plant TA proteins localize to a surprisingly wide variety of subcellular compartments including specific regions within the endomembrane system, mitochondria, chloroplasts, peroxisomes and the nucleus. For instance, one candidate Arabidopsis TA protein, proposed to be a member of the Tudor domain-containing Royal family of proteins and a homolog of the human BRCA2-interacting protein EMSY, localizes to novel nuclear regions where it may be involved in chromatin remodeling. Other novel candidate Arabidopsis TA proteins localize exclusively to the plastid; however, these proteins appear to employ different (N or C-terminal) molecular targeting signals to mediate their localization to plastids and the thylakoid subcompartment. These data as well as the results from experiments designed to examine the intracellular sorting mechanisms of one of the largest class of TA proteins, namely the SNARE proteins involved in vesicle transport within the endomembrane system, will be discussed.

2: Cloning and characterization of three Pathogenesis-Related Proteins in European plums

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Pathogenesis related (PR) proteins are induced by different stress conditions, especially after pathogen infection. Timely expression of these proteins is often associated with resistance to fungal diseases. Brown rot, caused by the *Monilinia fructicola* is the most serious fungal disease of stone fruits. This fungus attacks flowers causing blossom blight and also ripe fruits leading to serious economic loss. To date, the molecular mechanism of resistance to this fungal disease in stone fruits is unknown. We hypothesized that certain PR proteins could confer resistance to brown rot and explored four varieties of European plums (*Prunus domestica*) with varying degree of resistance to brown rot using SDS-PAGE of total proteins extracted from the fruits. Several proteins between molecular masses 15-30kD differed in their expression among the varieties. From these differentially expressed proteins, we cloned three PR proteins, PR-1, PR-5 and PR-10. Among these, PR1 and PR10 sequences were identical in the four varieties tested. However, PR5 protein showed significant differences in amino acid sequences, molecular mass and isoelectric point among the four varieties, suggesting that there is more than one isoform of PR5 in European plums. Currently, we are in the process of

purifying the various isoforms of PR5 proteins to test their antifungal activity and are also screening more varieties to confirm the variations in PR5. We expect that these variations may lead us to identify useful molecular markers to complement breeding. Concurrently, we are also trying to identify the regulatory elements controlling the expression of PR5, to understand the molecular mechanism involved in brown rot resistance in stone fruits.

3: Proteome analysis of soybean developing seed coats

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The relatively large seeds in soybean (*Glycine max*) make it a good legume model to study seed coat development. Soybean seed coats are an agricultural by-product that could be further utilized to produce high value proteins and augment the crop market value. Many seed coat proteins have been previously described and analyzed, but a comprehensive proteome analysis of seed coat tissues has not yet been reported. The identification of proteins and their allocation to the different seed coat maternal tissues (epidermis, hourglass cells and parenchyma) and filial tissue (aleurone) could provide insight to the seed coat function in the regulation of embryo development, seed biology, and open the possibility for manipulation of seed quality features such as seed size. We report here on a proteomic approach used to determine the protein expression profile of developing seed coats. Seed coat extracts were analyzed by 2D gels. In the early stage (10-20 days post-anthesis, DPA), the seed coat represents at least 87% of the seed mass and cell division is predominant, 724 protein spots were detected. In the mid stage of development (21-35 DPA) 811 spots were detected, corresponding to the elongation phase of seed development. In the late stage of development (36-50 DPA) 477 spots represent the accumulation of storage proteins as the seeds enter maturation. At late maturity (>70 DPA) only 130 spots were detected, because of the protein degradation that takes place as the seed desiccates. Two-dimensional maps of *cv* Harosoy 63 were analyzed by Image Master 2D Platinum 6.0 software. The expected dramatic changes in seed coat protein expression profile associated with the major developmental stages of the soybean seed are documented. Future work aims at elucidating key potential protein markers and potential developmental regulators.

4: Molecular mapping of genes involved in the phenylpropanoid pathway in bean (*Phaseolus vulgaris* L.)

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Previous genetic analyses identified 15 genes that control seed coat pattern and color in common bean. Many of these genes exhibit epistatic interactions with other genes, which define the many seed coat colors and patterns observed within the species. Some of these genes have been positioned on the common bean linkage map. It has been hypothesized that genes involved in the phenylpropanoid pathway correspond to some of the classical seed coat color genes in bean. In a previous study we cloned and sequenced fragments of thirty-five phenylpropanoid pathway genes from common bean. The purpose of the current work is to map the positions of the phenylpropanoid genes on the common bean linkage map and determine whether their position correspond to any of the loci for classical seed coat color genes. The mapping population that was used consisted of recombinant inbred (RI) lines derived from a cross between BAT 93 and Jalo EEP558. The parents for the population are also the parents of the core mapping population for *P. vulgaris* and their progeny show a range of seed coat colors. Polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLP) were identified for the phenylpropanoid gene sequences between parental lines. The segregation patterns of 18 phenylpropanoid pathway genes have been analysed in the RI population and their locations in the bean linkage map were determined by a MAPMAKER analysis. The additional genes in this pathway will be mapped in a similar way and cosegregation between phenylpropanoid and classical seed coat color genes will be tested.

5: The role of debranching enzymes in starch metabolism

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Plants produce carbohydrates through photosynthesis and store large amounts in the form of starch. This starch is used for growth (or re-growth) when photosynthesis is not possible. Starch is composed of polymers of the simple sugar glucose. The major polymer is amylopectin, in which chains of glucose molecules are connected to one another by branch points and arranged in such a way that the end product, the starch granule, has a semi-crystalline structure. At least three enzymes are believed to be directly involved in the synthesis of amylopectin, starch synthases (which make component chains), branching enzymes (which create the branch points) and, surprisingly, debranching enzymes (which cut the branch points). The involvement of the latter class has been revealed by examples of debranching enzymes-deficient plants which accumulate phytoglycogen, an altered glucose polymer. Phytoglycogen has an unusually high degree of branching, which prevents the formation of the semi-crystalline granule, but exact function of debranching enzymes in determining amylopectin structure is not yet well defined. The fact that the most known debranching enzymes knockouts do not show impaired in degradation is intriguing, as a complete degradation of starch requires the removable of existing branch points The aim of our research is to define precisely the how debranching enzymes are involved in the synthesis and degradation of starch. The Arabidopsis genome encodes four debranching enzymes. Using reverse genetics to knockout each gene we have created a full suite of single, double, and triple mutants together with the quadruple knockout. Analysis of the mutant phenotypes reveals functional specialisation for biosynthesis and degradation within the small gene family and illustrates the importance of starch metabolism in plant growth.

Notes

Session III: Abiotic stress
11:15 – 12:30 am, Rm: MDCL – 1105

1: Increased temperature during autumn conditions inhibits photosynthetic carbon gain in seedlings of *Pinus banksiana*

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Temperature and daylength act as environmental signals, which determine the length of the growing season in boreal evergreen conifers. Climate change might affect the seasonal development of these trees, as they will experience naturally decreasing daylength during autumn, while at the same time warmer air temperature will maintain photosynthesis and respiration. We characterized the downregulation of photosynthetic gas exchange and the mechanisms involved in the dissipation of energy in Jack pine (*Pinus banksiana*) in controlled environments during a simulated summer-autumn transition under natural and under conditions with altered air temperature and altered photoperiod. Using a factorial design we dissected the effects of daylength and temperature. Control plants were grown at either summer conditions with 16h photoperiod and 22°C (LD/HT), or conditions representing autumn with 8h/7°C (SD/LT). To assess the impact of photoperiod and temperature on photosynthesis and energy dissipation, plants were also grown under either cold summer (16h/7ºC; LD/LT) or warm autumn conditions (8h/22ºC; SD/HT). Photosynthetic gas exchange was affected by both daylength and temperature. Assimilation and respiration rates under warm autumn conditions were only about half of the summer values but were similar to values obtained for LD/LT and SD/LT treatments. In contrast, photosynthetic efficiency was largely determined by temperature, but not by daylength. Plants of different treatments followed different strategies for dissipating excess energy. Whereas in the LD/HT treatment safe dissipation of excess energy was facilitated via zeaxanthin, in all other treatments dissipation of excess energy was facilitated predominantly via increased aggregation of the light harvesting complex of photosystem II. These differences were accompanied by a lower de-epoxidation state and larger amounts of ß-carotene in the SD/HT treatment as well as by changes in the abundance of thylakoid membrane proteins compared to the summer condition. We conclude that photoperiod control of dormancy in *P. banksiana* appears to negate any potential for an increased carbon gain associated with higher temperatures during the autumn season.

2: The role of excitation pressure on the extent of variegation in the *immutans* mutant of *Arabidopsis thaliana*

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Variegation mutants are plants that develop sectors of different colours (green and white) in their vegetative parts. Variegation in the *immutans* mutant of *Arabidopsis thaliana* is induced by a nuclear recessive gene. The appearance of white sectors is sensitive to elevated temperature and high light intensities. White sectors result due to a blockage in carotenoid biosynthesis at the phytoene desaturation reaction. Phytoene desaturation is thought to require oxidized plastoquinone (PQ) as an electron acceptor. The oxidation of reduced plastoquinone (PQH_2) by the reduction of O_2 to H_2O is caused by an oxidase. IMMUTANS protein is presumed to be the oxidase that functions as a redox component in phytoene desaturase and also in the photosynthetic electron transport chain. It has been proposed that the lack of functional IMMUTANS results in over-reduction of the PQ pool, which leads to the inhibition of phytoene desaturase and the appearance of white sectors. Excitation pressure reflects changes in the redox state of the PQ pool and can by modulated by both light and temperature. Since the extent of variegation can be affected by changing the light and temperature, I hypothesized that the extent of variegation is regulated by excitation pressure. *immutans* plants were grown at 25ºC

and at 12ºC with increasing light intensities of 50, 150, and 450 umol $m^{-2}s^{-1}$. Leaf area measurements were performed using a dissecting microscope and the ratio of white to green sectors was analyzed, as well as the redox state of PQ pool by *in vivo* Chlorophyll *a* fluorescence. As the light intensity increased so did the extent of white sectors. An increase in the redox state of the PQ pool at both 25ºC and at 12ºC was also noted. Thus, it appears that the redox state of the PQ pool may regulate the extent of variegation.

3: High growth temperatures reduce photosynthesis, respiration and growth in black spruce

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The response of black spruce (*Picea mariana* (Mill.) B.S.P.) to predicted climate warming will have significant effects on the structure and functioning of the North American boreal forest. We investigated the growth, net CO_2 assimilation and dark respiration rates of black spruce seedlings grown at cool (22:15°C) and warm (30:23°C) temperatures. Cool-grown seedlings were taller and heavier than warm-grown trees and had significantly lower mortality. Photosynthesis of warm-grown seedlings was unable to acclimate, leading to reduced carbon assimilation at high temperatures. Dark respiration showed significant temperature acclimation, but the reduction in respiration was insufficient to compensate for the low photosynthetic rates in warm-grown seedlings. This study suggests that higher future temperatures will reduce the growth, survival and carbon uptake of black spruce.

4: Increase in specific proteins associated with the desiccation of tolerant roots of a horticultural dicot, *Ranunculus asiaticus*

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Ranunculus asiaticus is a horticultural dicot that is able to tolerate long-term desiccation during its annual life cycle. The annual cycle of desiccation and resurrection of *R. asiaticus* is correlated with the presence of a putative storage protein (JR14) and stress-related proteins (dehydrins and smHSPs) in its underground storage organs, the tuberous roots. SDS-PAGE analysis of all stages of its life cycle revealed that JR14 accumulated during the desiccation period, and two-dimensional electrophoresis demonstrated that there are two major proteins of around 14kDa. One of them was purified, and 31 N-terminal amino acids were determined by sequencing. A 489bp full length JR14 cDNA sequence was obtained by 3'-RACE and 5'-RACE PCR using oligonucleotide sequences based on the terminal amino acids. The amino acid sequence (163 amino acids) of the JR14 protein showed that it has stress-induced domains PLAT/LH2. A peptide of 24 N-terminal amino acids of the full length JR14 protein is a possible signal peptide, determined by comparison with the amino acid sequence of the mature protein. Western blots indicated that the expression of dehydrin Dhn4B (LEAII D-11 family) is induced by dehydration and ABA, and declines during rehydration. The cDNA sequence of *Dhn4B* has a very high similarity with known dehydrins in other species. As with dehydrins, smHSPs were also detected by western blots in the tuberous roots during desiccation, and their expression was induced by high temperature and dehydration.

5: Metabolic responses of *Thellungiella salsuginea* during acclimation to osmotic stress imposed under field or growth chamber conditions

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Thellungiella salsuginea (Yukon ecotype) is a sub-arctic crucifer that is highly tolerant of extreme environmental conditions. Using gas chromatography/mass spectrometry we identified stress-responsive changes among polar metabolites extracted from leaves of Thellungiella grown in controlled environment cabinets and subjected to cold temperature (5°C), simulated drought, or irrigation with water containing up to 500 mM NaCl. We also compared these profiles with those prepared from plants harvested at field sites in the Yukon. We hypothesize that metabolic traits essential for acclimation and stress tolerance in plants subjected to calibrated stress treatments in growth cabinets should also be among the traits expressed in plants exposed to the combination of natural temperature and osmotic stresses in the field. Of over 300 peaks in each chromatogram we identified 60 mass spectral components in profiles of chamber-grown plants that increased with exposure to stress: 5 were changes common to cold, salinity and drought while 13 appeared with two of the three stress treatments used. Of the 60 stress-responsive components, 27 (46%) were also higher in leaf extracts of Thellungiella growing on salt flats in the Yukon relative to control plants in cabinets. In general, field plants had more components associated with salt-or drought-treated plants in chambers as opposed to cold-associated metabolites, an observation consistent with the warm temperatures, saline soils and drought conditions experienced by the field plants we obtained. Thus comparisons between metabolic profiles from chamber and field grown plants can be a valuable experimental approach to reveal potentially important stress-responsive patterns from amongst the large datasets typically generated by “omics” technologies.

Notes

Session IV: Anatomy and Development
3:00 – 5:00 pm, Rm: MDCL – 1110

1: *GAMYB* gene expression and seed germination in tomato

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To understand the function of the GA transduction pathway during seed germination, the transcription factor gene *GAMYB*, which responds to the GA signal, has been studied in tomato (*Solanum lycopersicum*). We have identified two *GAMYB*-like genes in tomato, which show 83% amino acid similarity within their binding domain to barley, rice and *Arabidopsis GAMYB* genes. Both genes are present as single copies in the tomato genome. One *GAMYB*-like gene is expressed in both the embryo and endosperm during seed germination in *gib-1* mutant (non-GA producing) and wild-type seeds. Its transcripts are present in *gib-1* mutant seeds imbibed in water and are up-regulated by gibberellic acid (GA_3) during germination. *GAMYB*-like gene expression increases during germination and declines after it is completed in both intact *gib-1* mutant and wild-type seeds. However, dissected *gib-1* embryos germinate when imbibed in either water or GA, with almost no difference in the amount of mRNA transcribed by the *GAMYB*-like gene during this event. This is indicative that neither GA nor expression of the *GAMYB*-like gene are necessary for embryo germination but that they are involved in the germination of whole seed. The germination-inhibiting hormone ABA does not affect expression of this gene in the intact seed.

2: Developmental morphology of several vine members of the Cucurbitaceae

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Apical meristem development and subsequent branching of many Cucurbitaceae species is of interest due to the complex nature of the node; however few ontogeny studies exist to provide a morphological framework for investigation. This study provides a set of morphological characteristics by examining the ontogeny of several species of vine-forming Cucurbitaceae with similar growth habits but different tendril architecture. The floral development and phyllotactic patterns of the apical complex and shoot architecture were examined. Early ontogeny of the apical complex was determined using epi-illumination light microscopy and scanning electron microscopy. Results for *Sicyos angulatus* (L.) and *Ecballium elaterium* (L.) were compared to a similar study of *Echinocystis lobata* (Michaud). For all species examined, each leaf primordium has a complex axillary structure, offset from the leaf axil, which is not merely an axillary bud. This axillary complex undergoes a series of asymmetric divisions which give rise to structures in a set physical sequence: a male inflorescence, a female inflorescence, an axillary bud and a tendril except *E. elaterium* lacks the tendril. The axillary bud does not stay dormant and develops into either a compressed, quiescent shoot or continues growth to produce a branch. All 3 species have the same physical sequence of axillary structures. However, each species displays different timing patterns for the initiation and development of these structures. Some of these timing patterns appear to correspond to the pattern of production of male and female flowers.

3: Analysis of *Eriophorum vaginatum* corms, sclereid clusters, and adjacent substrate for 9 industrial metals related to mining in Sudbury, ON

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To examine metal deposition within sclereid clusters of *Eriophorum vaginatum*'s corm, the concentration of 9 metals (Al, Fe, Co, Ni, Cu, Zn, As, Cd, and Pb) was determined (by ICP-MS) for corms, sclereid clusters, and soil adjacent to the root zone, of plants growing in a contaminated and a reference site near Sudbury, ON (September 2005). Soil pH was similarly low at the contaminated and the reference site (3.72 and 3.38, respectively) suggesting metal availability in both sites was comparable. Metal concentrations of samples generally decreased in the order soil>corms>sclereid clusters. Sclereid cluster metal concentrations did not exhibit a relationship with site metal contamination. This is in contrast to soil and corm data sets which generally contained higher mean metal concentrations in the contaminated site than in the reference site. Principle Component Analysis (PCA) of the samples' metal concentrations also classified soil and corm samples by their respective sites, but did not likewise differentiate sclereid cluster samples. This suggests that metals, although largely excluded, can be taken into the plant body (i.e. the corm) but are not subsequently deposited into sclereid clusters, regardless of environmental contamination. That is, lignified sclereid clusters within *E. vaginatum*'s corm do not act as metal detoxification centres. However, these clusters contained more Fe and Al (~35-50 µg/g dry wt.) than any other tested metal by ~5 times. We propose Fe may be the cause of the red-orange colour of *E. vaginatum*'s sclereid clusters. Future work will examine the location and role of Fe and Al in sclereid clusters of *E. vaginatum*.

4: The role of abscission during leaflet separation in *Chamaedorea elegans* (Arecaceae)

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Leaf development in the palm family (Arecaceae) is unique and unlike any observed in other angiosperms. The objectives of this study were to follow leaf ontogeny in *Chamaedorea elegans* and to determine whether abscission plays a role in leaflet separation. Although previous studies indicated that abscission may be a mechanism by which leaflets separate, details of the process are lacking. The results of our ontogenetic study show that leaflet separation in *C. elegans* occurs in three steps that act together to produce the mature leaflets of a pinnate leaf. Young palm leaf primordia which have a hood-shaped appearance develop plications at an early stage of development through differential growth. The plications then separate and develop into leaflets through, first, schizogeny, in the direction of the abaxial ridge, which allows for subsequent mechanical rupture to take place. The cells at the fully separated leaflet margins show the deposition of protective properties, as the third step, prior to mechanical separation. Since these processes are present in 'typical' abscission, we propose that leaflet separation in *C. elegans* occurs by an 'abscission-like process'.

5: Ecotype screen for sequence and splicing variation at the MADS-AFFECTING FLOWERING LOCUS

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Mechanisms that control flowering time are predicted to display variation within individuals and across populations. Variability in expression of genes that control flowering time may acclimate reproductive development to unpredictable environmental conditions and variation across populations may segregate the flowering niche. Evidence suggests that the four tandemly quadruplicated MADS-Box genes at the *MADS-AFFECTING FLOWERING* (*MAF*) Locus, *MAF2* to *5*, display variability consistent with this prediction. *MAF* genes appear to be involved in flowering-time control similar to *FLOWERING LOCUS C* ,a homologue of the *MAF* genes, and natural variants may help to improve our understanding of *MAF* gene function. A transposition-like movement of the alternatively spliced section of *MAF3* into *MAF2* has created a fertile substrate for sequence change at *MAF2* by enhancing homologous recombination (HR) between *MAF2* and *MAF3*. The nine resultant allele groups are unusual as they have a distinct geographical signal. The series is associated with the eastern range of Arabidopsis thaliana, from the Alps to Central Asia and Northern Sweden. The center of variability suggests that Arabidopsis found refuge in the high altitude regions of Europe during a past glaciation event. Lack of novel variants outside of the center of diversity suggests that conditions present during the putative glaciation event fostered HR at this locus. My work has also established the *MAF* genes, both in WT and mutant forms in the HR series, are responsive to cold conditions via alternative splicing. Experiments are being conducted to examine the mechanism of mutation at *MAF2*, the phenotypic effect of the HR series of mutations, and to identify and test the modern descendants of the refugial populations for stress tolerance attributes.

6: Tissue specific accumulation of cyanogens and dynamics of cyanide levels in peach, plum and ornamental *Prunus spp*.

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Cyanogens, namely, cyagenic glucosides, cyanohydrins and hydrogen cyanide (HCN), are well-known compounds which are toxic for aerobic organisms. In mammals, ingested HCN levels as low as 0.5 mg $[CN^-]$ kg^{-1} bodyweight are generally toxic. Over 2650 plant species distributed throughout 130 families of angiosperms, gymnosperms, and ferns release large quantities of HCN upon tissue disruption or infection by a pathogen. The most studied cyanogenic plants are cassava, sorghum, barley and some *Prunus* species, which have been characterized in terms of their cyanogenic potential, a total amount of $[CN^-]$ released by plant tissues, and some steps of biosynthesis and catabolism of cyanogens. In *Prunus* species, investigations of cyanogens mainly associated with accumulation of cyanogenic glucosides in seeds. For example, in almond, it has been shown that the bitterness of kernels is defined by levels of amygdalin. On the other hand, in oriental medicine, extracts from apricot seeds have been widely used to treat asthma, aplastic anemia and tumors, in particular, amygdalin is stated as the major effective compound. Amygdalin represents a diglucoside catabolized by amygdalin hydrolase, to prunasin, a monoglucoside, mainly found in vegetative tissues. The purpose of the present study was to characterize major cyanogenic compounds and estimate the cyanogenic potential and dynamics of cyanide levels in several *Prunus* species, such as peach, plum and ornamental *Prunus spp*., grown in different conditions. It has been identified that prunasin is the major cyanogenic compound present in leaf and stem tissues in plum, peach and ornamental *Prunus*. Interestingly, both peach and plum contained amygdalin as well as prunasin in cotyledons and developing embryo tissues. It seems to be that biosynthetic steps and accumulation of the cyanogens are specific for a particular *Prunus* species. The preliminary data will be used for further characterization of the biosynthesis and catabolism of cyanogens in *Prunus spp.*

7: Identification and location of a candidate gene controlling peach fruit fuzz

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Peach fuzz is the main trait that differentiates peach and nectarine. Earlier studies show that this trait is controlled by a single gene, located on 41-45cM of the chromosome five in a *Prunus* reference map (Dirlewanger *et al* 1999). We hypothesized that peach fuzz development is similar to trichome pathway in *Arabidopsis*. Based on this hypothesis, we mined the peach genome with primers designed to amplify homologous genes involved in this pathway in *Arabidopsis*. Thus, we cloned the homologues of *TRANSPARENT TESTA GLABRA1 (TTG1), TRANSPARENT TESTA GLABRA2 (TTG2), GLABRA3 (Gl3),* and *GLABRA2 (Gl2)* in peach. The PCR products from the genomic DNA did not reveal any obvious differences in these genes between peach and nectarine. However, when BAC clone for the putative region controlling the fuzziness trait (41-45cM of Chr.5) of peach (Abbott, Clemson University) was amplified with the primers specific for TTG1, TTG2, GL3 and GL2, a fragment corresponding to the size of TTG1 in peach genomic DNA was revealed. In order to make sure that these two fragments are the same, they were digested with a restriction enzyme (*Ecor*I) that cuts within TTG1. The digestion patterns for the fragments amplified from peach genomic DNA and BAC clone plasmid DNA were identical. We further confirmed this by sequencing the fragments, which turned out to be the same. Our results confirm that a homologue of TTG1 is located in the 41-45 cM region of chromosome 5 in peach. This result increases the possibility that TTG1 homologue may be the main gene involved in peach fruit fuzziness. Currently we are cloning the full length TTG1 homologue in peach by genome walking from both peach and nectarine for further characterization.

Notes

Notes

Session V: Signaling pathways
3:00 – 5:00 pm, Rm: MDCL – 1102

1: Characterisation of the PERK family of receptor kinases: an emerging cytoskeleton connection

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Receptor kinases play a vital role in both plants and animals for intercellular signalling. Plants are predicted to contain over 400 receptor kinases although there is currently very little known about their specific biological functions. We originally identified PERK1 as a novel receptor kinase in *Brassica* and homology searches within the Arabidopsis genome led to the identification of 15 new members of the PERK gene family. The PERK gene family represents a novel class of receptor kinases which contain a proline rich, extracellular domain similar to extensins, structural proteins of the plant cell wall. Expression analysis of each of the PERK genes reveals two predominant patterns, those that are ubiquitously expressed throughout all plant tissues and those that are expressed specifically in the pollen. The aim of our current work is to investigate the biological function of the PERK family including analysis of mutant phenotypes and identification of downstream signaling partners. Antisense downregulation of PERK expression in Arabidopsis resulted in a range of different mutant phenotypes indicating the importance of this gene family in plant development (Haffani et al 2006, *Plant Signaling and Behaviour* 1: 251-260). Arabidopsis T-DNA knockout lines have since been identified for each of the PERKs although no visible mutant phenotypes have been observed in the single knockouts indicating considerable genetic redundancy between family members. We have produced a number of PERK double and triple mutants and are currently screening for chemical response phenotypes. Recent results show that some of the mutant lines display an increased sensitivity to the actin depolymerising drug Latrunculin B and the vesicle transport inhibitor Brefeldin A. In addition, yeast-two-hybrid screens have identified a number of potential interacting partners including a novel protein kinase KIPK2 (KCBP interacting protein kinase 2). We have shown that KIPK2 in turn interacts with the Arabidopsis Kinesin like Calmodulin Binding Protein (KCBP) which functions as a microtubule motor protein. These results are painting an emerging picture of the PERKs potential role in cytoskeleton associated vesicle transport.

2: A mutation in *LINES*, which encodes a RING-type ubiquitin ligase, disrupts *Arabidopsis* adaptability to nitrogen limitation and alters the responsive transcriptiome

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Abundant nitrogen is required for plant growth and development, while numerous biotic and abiotic factors that consume soil nitrogen frequently create a nitrogen limitation growth condition. To cope with this, plants have evolved a suite of nitrogen limitation adaptive responses. However, the molecular mechanism governing plant adaptability to nitrogen limitation is totally unknown because no reported mutant defines this trait. Here, we isolated an *Arabidopsis* mutant, *lines* (*low inorganic nitrogen-induced early senescence*), and identified the *LINES* gene as an essential component in this molecular mechanism. Supplied with insufficient inorganic nitrogen, the *lines* mutant failed to develop the essential nitrogen limitation adaptive responses, but senesced much earlier and more rapidly than did wild type. Under other stress conditions including low phosphorus nutrient, drought and high temperature, the *lines* mutant did not show this early senescence phenotype. Map-based cloning of *LINES* revealed that this gene encodes a RING-type ubiquitin ligase. The LINES protein is localized to the nuclear speckles, where this protein interacts with the *Arabidopsis* ubiquitin conjugase 8 (AtUBC8). In the *lines* mutant, the RING domain was deleted from LINES, which resulted in alteration of LINES subcellular localization, disruption of the interaction between LINES and AtUBC8, and the

low inorganic nitrogen-induced early senescence phenotype. Microarray analysis revealed that nitrogen limitation significantly changed the transcription of 618 genes with 330 up-regulated and 288 down-regulated in *Arabidopsis* plants. The up-regulated group included the genes for protein degradation and the anabolism of anthocyanin and phenylpropanoids. The down-regulated group contained the genes functioning in photosynthesis and in the synthesis of nitrogenous macromolecules such as chlorophyll, proteins, amino acids and nucleotides. The absence of the functional *LINES* gene in *Arabidopsis* plants extensively and markedly altered their responsive transcriptome to nitrogen limitation. The *lines* mutant had a total number of 1799 genes up- or down-regulated by nitrogen limitation, about 3-fold of that in wild type.

3: Islands of co-expressed neighbouring genes in *Arabidopsis thaliana* suggest global regulation of chromatin structure

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Biochemical and cytogenetic experiments have led to the hypothesis that eukaryotic chromatin is organized into a series of distinct domains that are functionally independent. Two expectations of this hypothesis are: 1) adjacent genes are more frequently co-expressed than expected by chance, and 2) co-expressed neighbouring genes are often functionally related. We have found that over 10 percent of *Arabidopsis thaliana* genes are within large, co-expressed chromosomal regions. Two percent (497/22,520) of genes are highly co-expressed (r>0.7), about five times the number expected by chance. These genes fall into 226 groups distributed across the genome, and each group typically contains two to three genes. Among the highly co-expressed groups, forty percent (91/226) have genes with high amino acid sequence similarity. Nonetheless, duplicate genes alone do not explain the observed levels of co-expression. Co-expressed, non-homologous genes are transcribed in parallel, share functions, and lie close together more frequently than expected. Our results show that the *A. thaliana* genome contains domains of gene expression. Small domains have highly co-expressed genes that often share functional and sequence similarity and are likely co-regulated by nearby regulatory sequences. Genes within large, significantly correlated groups are typically co-regulated at a low level, suggesting the presence of large chromosomal domains. The frequency of neighbouring genes in convergent orientation is lower than expected. This may suggest that convergent gene pairs are associated with siRNA-mediated mRNA degradation.

4: The Arabidopsis TGA2 repressor binds the co-activator NPR1 to form an SA-regulated enhanceosome

Amanda Rochon[1], Patrick Boyle[1], Tracy Wignes[2], Pierre R. Fobert[2] and Charles Despres[1]
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NPR1 is a key protein required for systemic acquired resistance (SAR). In untreated wild-type Arabidopsis, endogenous NPR1 is present in both the cytoplasm and the nucleus. However, when overexpressed as a GFP fusion in the *npr1-1* mutant background, NPR1-GFP is sequestered inside the cytoplasm, in untreated cells. This sequestration is due to the oligomerization of NPR1-GFP, resulting from disulfide bridges between cysteines 82 and 216. Treatment of Arabidopsis plants overexpressing the NPR1-GFP fusion protein with INA (2,6-dichloroisonicotinic acid, a salicylic acid (SA) analogue), reduced the cysteines and caused NPR1-GFP to monomerize allowing its nuclear localization. Once inside the nucleus, NPR1 interacts with TGA transcription factors resulting in the activation of defense genes, such as *PR-1*, and in the deployment of SAR. Although the interaction between NPR1 and TGA factors is well established, the mechanism by which they regulate gene expression and deployment of SAR is unclear. Our current results show that both TGA2 and NPR1 are recruited to *PR-1* independent of each other and of SA treatment. Consistent with the result that a triple knockout in *TGA2/5/6* derepresses *PR-1*, *in vivo* plant transcription assays revealed that TGA2 is not an autonomous transcription activator but is a transcriptional repressor in both untreated and

SA-treated cells. However, after stimulation with SA, TGA2 is incorporated into a transactivating complex with NPR1 forming an enhanceosome. These data further our understanding of the mechanism by which TGA2 and NPR1 activate *PR-1*.

5: The NPR1 BTB/POZ domain is required for both TGA2-dependent NPR1 coactivator function and PR-1 gene induction

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The NPR1 protein is the chief regulator of systemic acquired resistance (SAR). The current model for the deployment of SAR postulates that following treatment with salicylic acid (SA) C82 and C216 of NPR1 are reduced, liberating the protein from a cytoplasmic oligomer and permitting its translocation to the nucleus. Within the nucleus NPR1 interacts with TGA transcription factors to induce defense genes, including the SAR marker P*athogenesis-Related-1* (*PR-1*) gene, ultimately culminating in the establishment of SAR. The NPR1 protein possesses two protein-protein interactions motifs: An N-terminal BTB/POZ domain and ankyrin repeats. While the function of the NPR1 BTB/POZ domain in disease resistance has yet to be determined, the ankyrin repeats are known to mediate interactions with the TGA transcription factors, since mutations affecting this motif abrogate NPR1-TGA interaction, *PR-1* gene activation and the deployment of SAR. To address the role of the NPR1 BTB/POZ domain we generated a series of mutants specifically targeting structural elements essential to the folding and function of the promyelocytic leukemia zinc finger (PLZF) BTB/POZ domain, the archetypical BTB/POZ. *In vivo* plant two hybrid assays indicated that the NPR1 BTB/POZ domain contributes but is not essential for NPR1-TGA2 interaction. However *in vivo* transcription assays revealed that the core of the BTB/POZ domain is required for TGA2-NPR1 mediated transactivation and proteins compromised in these elements were unable to complement *PR-1* gene expression in the npr1-3 mutant background. Thus both biochemical and genetic data demonstrate that the transactivation of the TGA2-NPR1 enhanceosome is contingent upon the presence of core elements of the NPR1 BTB/POZ domain.

6: The chimeric cyclic nucleotide-gated ion channel AtCNGC11/12 induces programmed cell death

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The hypersensitive response (HR) is a controlled death of cells that develops in plants in response to pathogen infection. A number of *Arabidopsis* mutants, which spontaneously develop HR-like cell death, have been identified; they are referred to as lesion mimic mutants. We isolated one of these mutants, *cpr22*, which shows constitutive activation of pathogen resistance responses. The *cpr22* mutant has a 3kb deletion within a cluster of cyclic nucleotide gated ion channel (CNGC) genes resulting in the creation of a novel chimeric CNGC gene (ATCNGC*11/12*). The phenotype conferred by *cpr22* is attributable to the expression of *ATCNGC11/12*. In this study, we conducted a comprehensive analysis of the cell death induced by transient expression of *ATCNGC11/12* in *Nicotiana benthamiana.* In cells expressing *ATCNGC11/12*, a number of morphological characteristics specific to programmed cell death (PCD) were observed, including plasma membrane shrinkage, membrane blebbing and the maintenance of intact organelles. Fragmentation of chromatin DNA which is a hallmark of animal PCD was also observed using the TUNEL assay. Recently, *vacuolar processing enzyme* (VPE) was isolated as a first plant caspase-like protein, which is involved in pathogen inducible HR development. To investigate the effect of VPE, *VPE*-silenced *N. benthamiana* was created. In *VPE*-silenced plants, HR development by transient expression of *ATCNGC11/12* was much slower and weaker compared to control plants, suggesting the involvement of this plant caspase. Complementation analysis using yeast mutants demonstrated that the *ATCNGC11/12* channel is permeable to Ca^{2+}. Furthermore, the development of HR by *ATCNGC11/12*

expression can be inhibited by addition of calcium channel blockers such as $GdCl_3$. Taken together, these results indicate that the cell death that develops in the *cpr22* mutant is indeed PCD and that the chimeric channel, ATCNGC11/12, is at the point of, or up-stream of the calcium signal necessary for the development of HR.

7: Identification of a functionally essential amino acid for the cyclic nucleotide gated ion channel ATCNGC11/12

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To investigate the resistance signaling pathways activated by pathogen infection, we previously identified the Arabidopsis mutant constitutive expresser of PR genes22 (cpr22) (Yoshioka et al., 2001). The cpr22 mutation was identified as a 3-kb deletion that fuses two cyclic nucleotide-gated ion channel (ATCNGC)–encoding genes, ATCNGC11 and ATCNGC12, to generate a novel chimeric gene, ATCNGC11/12 (Yoshioka et al., 2006). Genetic, molecular, and complementation analyses suggest that ATCNGC11/12, as well as ATCNGC11 and ATCNGC12, form functional cAMP-activated CNGCs and that the phenotype conferred by cpr22 is attributable to the expression of ATCNGC11/12. Recently, a genetic screen for mutants that suppresses cpr22-conferred phenotypes identified one intragenic mutation of cpr22 (suppressor #73). This mutation (E527V) locates within the eighth -strand of the cyclic nucleotide binding domain in ATCNGC11/12. The mutant suppressor #73 is morphologically identical to wild type and lost spontaneous cell death formation and enhanced pathogen resistance. Transient expression in Nicotiana benthamiana confirmed that this mutation E527V is responsible for suppression of spontaneous cell death formation by ATCNGC11/12. Furthermore, heterologous expression using a K^+ uptake deficient yeast mutant revealed that this amino acid change causes suppression of K^+ channel function of ATCNGC11/12. Computational structural modeling suggests that E527 is a key residue for stability of channel formation and therefore function of this CNGC.

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Session VI: Respiration, Heavy metals, Phytoalexins and Pathogens
3:00 – 5:00 pm, Rm: MDCL – 1105

1: Mesocarp localization of a bifunctional resveratrol/hydroxycinnamic acid glucosyltransferase of Concord grape (*Vitis labrusca*)

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Resveratrol is a stilbene with well-known health-promoting effects in humans and is produced constitutively or accumulates as a phytoalexin in several plant species including grape (*Vitis* sp.). Grape berries accumulate stilbenes in the pericarp as *cis*- and *trans*- isomers of resveratrol together with their respective 3-*O*-monoglucosides. An enzyme glucosylating *cis*- and *trans*- resveratrol was purified to apparent homogeneity from Concord (*Vitis labrusca*) grape berries and peptide sequencing associated it to an uncharacterized *Vitis vinifera* full-length clone (TC38971, TIGR database). A corresponding gene from *Vitis labrusca* had 98% sequence identity to clone TC38971 and 92% sequence identity to a *Vitis vinifera p*-hydroxybenzoic acid glucosyltransferase that produces glucose esters. The recombinant enzyme (VLRSGT) was active over a broad pH range (5.5 to 10) producing glucosides of stilbenes, flavonoids and coumarins at higher pH and glucose esters of several hydroxybenzoic and hydroxycinnamic acids at low pHs. *Vitis labrusca* grape berries accumulated both stilbene glucosides and hydroxycinnamic acid glucose esters that are consistent with the bifunctional role of VLRSGT in stilbene and hydroxycinnamic acid modification. While phylogenetic analysis of VLRSGT with other functionally characterized glucosyltransferases places it with other glucose ester producing enzymes, the present results broaden the biochemical activities of this class of enzymes.

2: An *Arabidopsis* glyoxylate/pyruvate-dependent GABA transaminase

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Gamma-aminobutyrate (GABA) is a ubiquitous non-protein amino acid that has been implicated in stress metabolism and signaling in plants. GABA transaminase (GABA-T) catalyses the catabolic breakdown of GABA to succinic semialdehyde, and both pyruvate- and 2-oxoglutarate-dependent activities are reported in the literature. In this report, we further characterized a recombinant *Arabidopsis* GABA-T using enzyme-linked assays to determine the formation or disappearance of reducing equivalents, and HPLC to determine the formation of amino acid products. Removal of the hydrophobic mitochondrial signaling peptide improved the total recovery of recombinant enzyme and increased specific activity by 2-fold without altering specificity. The recombinant enzyme exhibited maximal activity at pH 9, and utilized both glyoxylate and pyruvate, but not 2-oxoglutarate, with physiologically-relevant affinities. Both pyruvate- and glyoxylate-dependent activities were present in cell-free extracts from wild-type plants, but were diminished or lost in GABA-T knockout mutants. The catalytic lysine residue, lysine 327, was identified with site-directed mutations that prevented Schiff base formation and removed all detectable enzymatic activity, even though there was no impact on protein folding as determined by equilibrium denaturation analysis. These data confirm that the novel cDNA encodes an enzyme responsible for the catabolism of GABA *in planta*, and suggests that GABA metabolism interacts with the photorespiratory pathway.

3: Functional analysis of an Arabidopsis succinic semialdehyde reductase

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Exposure of plants to various biotic and abiotic stresses results in the decarboxylation of glutamate and the accumulation of γ-aminobutyric acid (GABA). The catabolism of GABA occurs via pyruvate or α-ketoglutarate-dependent GABA-transaminases to succinic semialdehyde (SSA), which in turn, is either oxidized to succinate via NAD^+- dependent succinic semialdehyde (SSADH) or reduced to γ-hydroxybutyrate (GHB) via NAD(P)H-dependent reductases. Our laboratory recently used a yeast complementation strategy to identify a novel *Arabidopsis* cDNA, which when reintroduced into a SSADH-deficient yeast enables growth on GABA and enhances the GHB level by 8-fold. In this study, biochemical analysis revealed that the recombinant enzyme is bifunctional, using either SSA (designated as SSA reductase or SSAR) or glyoxylate as substrate in an irreversible NADPH-dependent reaction. To investigate the SSAR function of this enzyme *in planta*, wild-type *Arabidopsis* and two loss-of-function mutants were subjected to submergence stress and gene expression was assessed by real-time PCR and metabolite analysis, as well as NADPH/$NADP^+$ ratios and lipid peroxidation over a 6-h time course. Transcript and GHB levels increased over time in wild-type plants, but not in mutants. The NADPH/$NADP^+$ ratio also increased in the wild type, but lipid peroxidation was only marginally affected, if at all. The NADPH/$NADP^+$ ratio in the mutants increased less than expected and even though lipid peroxidation was higher than in the wild-type plants under non-stress conditions, lipid peroxidation actually declined under stress. These data confirm that the cDNA encodes an enzyme responsible for the production of GHB in plants and suggests that even though SSAR activity contributes to redox homeostasis under submergence, other compensatory mechanisms exist in the cell to minimize oxidative damage.

4: Dynamics of arbuscular mycorrhizal symbiosis in heavy metal phytoremediation

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Increasing environmental pollutants, such as heavy metals (HM), pose significant threats to ecosystems and human health. Through a process known as phytoremediation, plants are used to remove these pollutants from contaminated environments. Moreover, the arbuscular mycorrhizal (AM) symbiosis, an ancient interaction between plant roots and Zygomycetes fungi, is recognized for benefiting host plants subjected to stress factors, including soil-HM toxicity. Hence, our study investigated the roles of the AM symbiosis in HM phytoremediation using experimental and meta-analytical approaches. We have determined that the AM fungi provide (1) an increased HM phytoextraction via 'Enhanced Uptake' at low soil-HM levels, and (2) a reduced HM bioavailability via 'Metal-Binding' processes at high soil-HM levels then resulting in increased plant biomass. This 'Dynamic Mycorrhizal Uptake' model further suggests that AM fungi, by buffering toxic HM conditions for the host plant, enhance plant HM tolerance via stress-avoidance.

5: Analysis of RNA silencing suppressor activity by TGBp1 of *Pepino Mosaic Virus*

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Virus-induced gene silencing (VIGS), a process that involves the targeting and subsequent degradation of viral RNA by a sequence-specific siRNA, protects plants from extensive virus infection. Plant viruses encode suppressors of the silencing process as a counter defensive strategy. The goal of the presented research was to determine whether the triple gene block protein 1 (TGBp1) of *Pepino mosaic potexirus* (PepMV) is such a suppressor. To induce silencing, constructs encoding green fluorescent protein (GFP) or β-glucuronidase (GUS) were introduced by agroinfiltration into leaves of *N. benthamiana* transgenic for *GFP* or *N. tobacum* transgenic for *GUS*. Co-infiltration with *Potato virus X* TGBp1 and PepMV TGBp1 restored GFP- respectively GUS- expression in systemic but not in local, infiltrated leaves to a similar extent. Analysis of GFP transgenic plants showed that this suppression of silencing was restricted to areas around the veins of the systemic leaves. Several lines of TGB1-transgenic tomato were generated and examined for phenotytpe. MicroTom tomato expressing TGBp1 showed mild developmental abnormalities at ambient temperatures. More severe abnormalities, similar to Pepino mosaic disease symptoms, were observed after transfer to 15^0C. PepMV infection of non-transgenic and transgenic plants led to the development of symptoms ranging from browning of leaves to death. A higher percentage of infected TGB1-transgenic plants died compared to infected non-transgenic plants. Together these results suggest that PepMV TGBp1 is a suppressor of systemic silencing. Because *TGB1*-transgenic plants show symptoms that increase at lower temperature it is suggested that TGBp1 also suppresses the silencing by a miRNA of a mRNA involved in normal plant development.

6: Taxonomic distribution of alternative oxidase and plastoquinol terminal oxidase in all kingdoms of life

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Alternative oxidase (AOX) is a mitochondrial inner membrane protein present in plants that introduces a branch point in the electron transport chain (ETC). AOX bypasses two of the three sites of proton translocation from the matrix to the intermembrane space and therefore impacts the energetic efficiency of respiration. Given the apparently wasteful nature of AOX in terms of energy conservation, effort has been put toward understanding the function of this pathway. Plastoquinol terminal oxidase (PTOX) is a related protein found in chloroplasts that creates a branched photosynthetic ETC.
As of 2000, the known distribution of AOX was limited to a select few eukaryotic lineages, namely plants, fungi, and protists, while PTOX was limited to green algae and higher plants. I used a bioinformatics approach to conduct sequence similarity searches of molecular databases to identify novel AOX and PTOX sequences and examined the taxonomic distribution of AOX and PTOX in nature. Both AOX and PTOX sequences were recovered in groups of organisms where they had not been identified previously. AOX was found for the first time in prokaryotes, animals, and a number of other organisms while PTOX was found in a several prokaryotes (namely cyanobacteria) and eukaryotes capable of oxygenic photosynthesis. This work shows that AOX and PTOX have broad taxonomic distributions and has led to the development of a hypothesis on the origin and evolutionary history of these proteins.

7: Molecular approaches toward improving *Fusarium graminearum* resistance in corn

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Fusarium graminearum causes gibberella ear rot in corn. Commercial corn hybrids have little or no resistance to infection by *F. graminearum*. The incidence and severity of the disease is strongly influenced by environmental factors and can reach epidemic levels, with losses of millions of dollars to Canadian farmers. The infection can result in severe reductions in crop quality because of contamination of the grain with trichothecene mycotoxins, such as deoxynivalenol. Because trichothecenes are resistant to high cooking temperatures as well as chemical methods of destruction they can accumulate to high levels in grain-based foods. In feed corn, mycotoxins cause a wide array of ailments in swine. The objective of this research is to identify the genes underlying the previously identified Quantitative Trait Loci (QTL) for resistance to gibberella ear rot (caused by *F. graminearum*) in corn. Because the genomic regions included in QTL are large enough to potentially encode hundreds of genes and resistance to Fusarium is complex, different genomic approaches (EST mapping, microarray) are used to identify resistance genes for the disease. Based on available information for genes/sequences associated with the Fusarium resistance in cereals, 250 sequence-specific PCR primers were designed and screened with the parental DNA (CO387, resistant and CG62, susceptible). Screening of the recombinant inbred (RI) population and subsequent mapping is underway. The identified resistance genes will be converted to markers that can easily be scored to allow rapid introgression into elite germplasm. Corn hybrids with resistance to Fusarium would reduce the incidence of fungal toxins in corn, thus leading to a safer food stream and preventing feed refusal by animals. Knowledge of the resistance mechanism could also be exploited to increase resistance by augmenting the affected pathway through chemical means.

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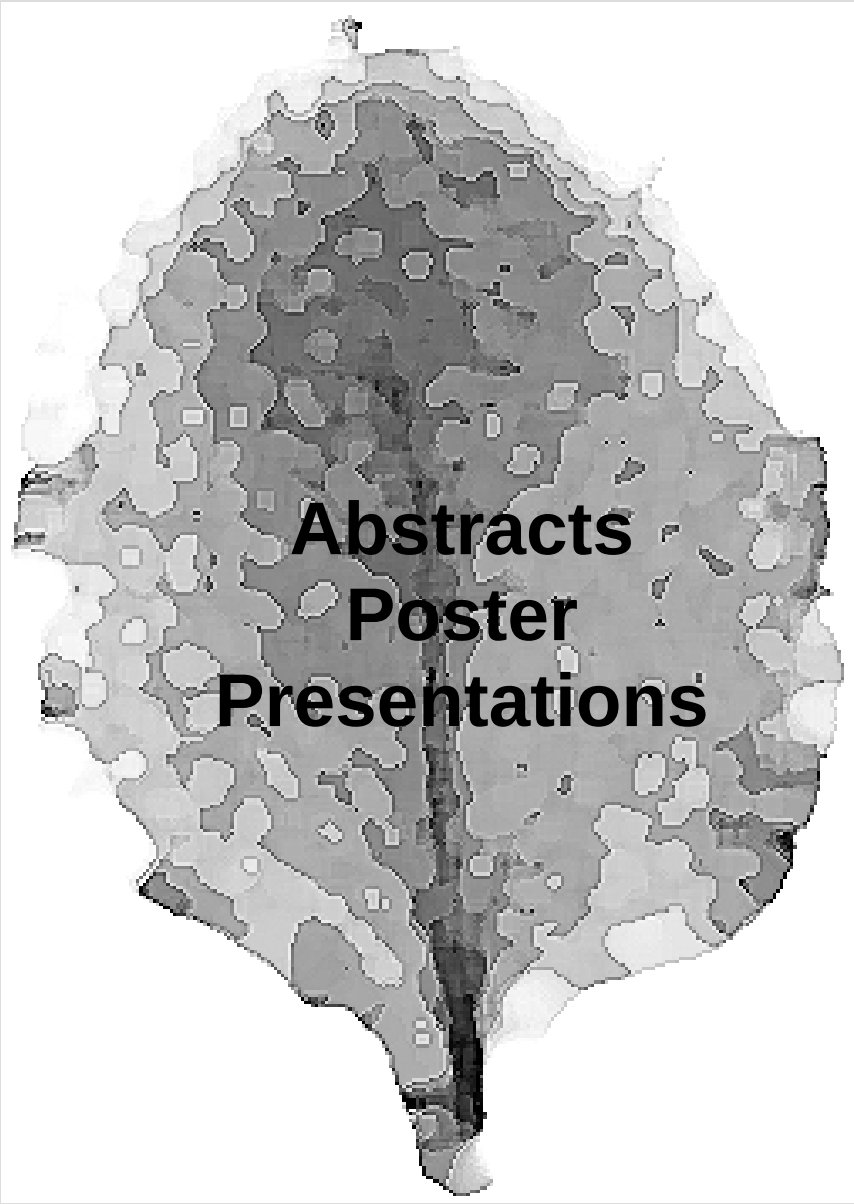

Abstracts Poster Presentations

12:30 – 2:00 pm, Rm: MDCL – South Hallway

P1: The role of *IAP1* in age-related resistance

Jessie Carviel and Robin K. Cameron
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Many plant species possess defense pathways such as Age-Related Resistance (ARR). ARR has been observed in numerous plant species, the cardinal feature being that disease resistance increases as the plant matures. *Arabidopsis* mutants which do not display ARR to *Pseudomonas syringae* pv. *tomato* (*Pst*) are currently being used as tools in an attempt to elucidate the ARR pathway. A mutant, *iap1-1*, (important in the ARR pathway,) is defective in the ARR pathway and was obtained through classical genetic screening. This mutant was assayed for the ability to exhibit ARR by challenging with virulent *Pst* and quantifying bacterial growth *in planta* at young and mature time points. The *iap1-1* mutant was ARR-defective, indicating that the wild type *IAP1* gene is important for ARR function. Testing was done to determine if basal resistance, (early disease resistance seen in young plants,) as well as SAR was also affected by the mutation. Young *iap1-1* plants displayed a normal basal resistance response to *Pst* and the SAR pathway appears to be intact, suggesting that the mutation is not involved in these pathways. Salicylic acid (SA) is required for the ARR response and is thought to be one of the end products of the ARR pathway and previous data suggests that it may act as an anti-microbial agent during ARR. Mutations in the *EDS1* gene, which functions upstream of SA in defense signaling abolish ARR. Intercellular addition of SA to *iap1-1* plants improved resistance two-fold, suggesting that intercellular SA accumulation has been impaired by this mutation and that wild type *IAP1* lies upstream of SA accumulation during ARR. We are in the process of mapping *iap1-1* using cleaved amplified polymorphic sequences. Identification and characterization of *iap1-1* will provide important insights into the ARR defense pathway.

P2: Assessment of the metabolic activity of chamber-grown *Fragaria virginiana* during flowering

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Flowering is a time of increased metabolic stress in angiosperms. Gynodioecious plants, such as the wild strawberry (*Fragaria virginiana*) present a unique opportunity to study this, as the plants exist as either females or hermaphrodites. It is thought that hermaphroditic plants are under greater stress during flowering as the plant is obligated to meet the metabolic demands of both male and female flower parts. In the current study, we set out to assess this supposition. *F. virginiana* plants of known sex were maintained in a cold room at 4^{o}C for 2 months, then place in a growth chamber (20^{o}C , 16-hour day, 14^{o}C 8-hour night) to induce flowering. Plants were sampled before, during and after flowering. At each sampling, metabolic activity was assessed in two ways. The primary method was to determine the photosynthetic activity of the plant using the Li-Cor 6400 Portable Photosynthesis System. At each sampling, the photosynthetic rate of the largest leaf on ten female and hermaphrodite plants was measured. The secondary method was to assess the vesicular-arbuscular mycorrhizal (VAM) fungal density. VAM are a morphotype of the symbiotic mycorrhizal association common to many plants, including the wild strawberry. The authors hypothesized that a positive correlation between photosynthetic rate and VAM fungal density would exist. To measure VAM density, roots from the ten plants assessed for photosynthetic rate were cleared and stained with Trypan blue, rendering fungal structures quantifiable via microscopy. The observed correlations will be discussed in the poster presentation.

P3: Identification and characterization of *cis*-acting elements in *CHS7* and *CHS8* gene promoters in *Glycine max*

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Our previous study on transcriptome analysis during embryo development in soybean has revealed an important role of *CHS7* and *CHS8* genes in the synthesis of isoflavonoids. As an initial step towards understanding the molecular details of how these two genes may control the isoflavonoid synthesis, a combination of molecular biology and bioinformatics tools were used to identify the nucleotide sequence in the promoter region and its *cis*-regulatory elements. We cloned and sequenced two fragments; 1617 bp upstream of *CHS7* and 1556 bp upstream of *CHS8* by Genome walking procedure. Both TSSP and GENOMATIX programs were further applied to predict potential transcription factor binding site (TBS). A total of 71 potential TBSs within *CHS7* promoter region and 73 potential TBSs within *CHS8* upstream sequence were predicted and annotated. We found that 57 of the potential regulatory elements were shared by both genes. Ongoing studies are designed to demonstrate the functional interaction of candidate transcription factors with the *cis*-acting regions of *CHS7* and *CHS8* genes.

P4: Harvesting *Catharanthus* leaf epidermis proteins and mRNA by Carborundum abrasion technique: Molecular cloning and functional characterization of Epidermis specific 16-hytroxytabersonine-16-*O*-methyltransferase (16-OMT)

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The monoterpene indole alkaloid (MIA) vindoline, an essential moiety of the antineoplastic agents, vinblastine and vincristine, is formed from tabersonine through six sequential enzymatic steps in at least three different cell types. The *O*-methylation of 16-hydroxytabersonine is catalyzed by an S-adenosylmethionine (SAM) dependent *O*-methyltransferase (OMT) that has yet to be cloned and characterized. Previous attempts to isolate 16-OMT from *Catharanthus* instead purified a flavonoid OMTs from cell culture extracts that led to the biochemical characterization of a different flavonoid-specific 4'OMT (FOMT) (Schröder *et al*, Phytochemistry, 2003, 62:127). Studies using laser capture microdissection (LCM) and carborundum abrasion (CA) techniques have shown that 16-OMT is preferentially expressed in *Catharanthus* leaf epidermal cells whereas FOMT activity was restricted to internal leaf tissues (Murata, Plant J, 2005, 44:581). The unique use of CA technique for large scale extraction of leaf epidermis enriched proteins was combined with size exclusion, adenosine agarose affinity and anion exchange chromatography to purify 16-OMT to homogeneity that was devoid of contaminating FOMT activities. Nevertheless, peptide sequences obtained from 16-OMT had high sequence identity to known *Catharanthus* flavonoid OMTs (including FOMT). A novel functionally active & unique 16-OMT was cloned using appropriate primers based on slight differences in some peptide sequences and a unique cDNA library made with leaf epidermis-enriched mRNA. The 16-OMT clone has been functionally expressed in E. coli and has been shown by various biochemical and molecular techniques to be a highly specific16-hytroxytabersonine-16- *O*-methyltransferase preferentially expressed in leaf epidermis.

P5: *Catharanthus roseus* somatic embryo development and monoterpenoid indole alkaloid biosynthesis

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Catharanthus roseus (Madagascar periwinkle) produces a broad spectrum of monoterpenoid indole alkaloids (MIAs), many of which are significantly useful for their pharmaceutical applications. In particular, two dimeric MIAs, vinblastine and vincristine, have anti-neoplastic properties and have been used for cancer chemotherapy for more than three decades, and there are still demands for developing high MIA yielding varieties of this plant, since vindoline is not available from other sources. Here we report the establishment of highly efficient somatic embryogenesis of *Catharanthus roseus* that has been used to conduct metabolic and genomic profiling of vindoline biosynthesis at various stages of somatic embryo development. Our results clearly show that in the absence of light, the appearance of pathway intermediates, enzyme activities and gene expression in MIA biosynthesis is developmentally regulated. Early pathway enzymes are expressed after the embryos establish their epidermal cell layer, whereas later steps are more highly expressed with embryo maturation. When somatic embryos are exposed to light, this triggers expression of the late steps in vindoline biosynthesis as previously described for *Catharanthus* seedlings. Interestingly, somatic embryos also express an entirely novel, previously undescribed pathway that is also developmentally regulated and that may have important implications for the regulation of MIA biosynthesis. The results will be discussed in relation to the possible application of somatic embryos for the development of varieties with unique MIA profiles.

P6: Natural genetic variation for water stress responses in *Zea mays* roots

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Elite maize germplasm has variable responses to abiotic stress. Some genotypes yield well in a wide range of environments while other genotypes yield well in a small number of environments. As a model system to investigate the basis for variability among genotypes across environments, we are studying seedling root growth rates in response to water stress among a population of maize inbred lines. Root growth rates across different growth conditions vary in a genotype specific manner ($P<0.001$). Increased concentration of the amino acid proline in the root tip enhances a plant's osmotic adjustment, and there is a significant correlation between proline and root growth under osmotic stress. Analyses of root tips from twelve inbred lines indicate that proline concentrations can differ over 5-fold among lines under control growth conditions with a range from 8.13 (inbred line SD80) to 43.85 (CG73) micromoles per gram dry weight tissue ($P<0.001$). Although the amount of proline per dry weight increases in all genotypes under water stress conditions as compared with control conditions (ranging from 25% higher (CG108) to close to 300% higher (SD80)), some lines have lower proline concentrations under water stress conditions than others do under control conditions. Using 122 microsatellite loci distributed across chromosomes 1 through 10, we have identified linkage disequilibrium between root growth traits and loci within the maize genome. We are examining expression profiles across lines and conditions to determine if genetic variation for expression profiles is as great as the genetic variation observed for physiological traits. Our results suggest that a comprehensive understanding of plant responses to environmental change must take into account plant genotype.

P7: Transgressive segregation for herbivory resistance in *Brassica napus*

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Breeding and plant development studies have shown that two parents that differ little for a trait can have descendants that differ substantially for the same trait, a phenomenon termed transgressive segregation. We used rapeseed, *Brassica napus*, a target crop species of several major insects in Canada, to detect novel genetic variation for insect resistance among a population derived from two similar parents. We also investigated the genetic basis for this resistance. A double haploid (DH) population was developed from microspores of the F1 hybrid between two *B. napus* DH lines, one with *Sinapis alba* germplasm introduced by interspecies cross. We measured the weight gain of cabbage white (*Pieris rapae*) larva that fed on the parental and double haploid plants in greenhouse, no-choice feeding experiments. Weight gain of cabbage white larvae did not significantly differ between parents but did differ among DH lines. Similarly, no-choice feeding experiments with the generalist herbivore, the cabbage looper (*Trichoplusia ni*), found significant genotypic differences among DH lines but no significant parental differences. Novel, transgressive lines were also found in field studies. For each member of the DH population, damage from flea beetle (*Phyllotreta* ssp.) at different plant development stages was evaluated at the Elora Research Station from May to July in 2004 and repeated in 2005. Again, plant damage between parents did not significantly differ, and members of the DH population did. To identify the genetic elements that explain the differences among lines, we surveyed we have surveyed 128 simple sequence repeat (SSR) microsatellite markers and 32 fluorescent amplified fragment length polymorphism (fAFLP) primer pairs across 118 DH lines. We have obtained over 1,900 DNA fragments from the SSR and fAFLP markers and identified 18 markers with significant effects on plant resistance to herbivores.

P8: Testing the usefulness of a cDNA microarray as a screen for high-isoflavone soybean lines

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Isoflavones are secondary metabolites, produced mainly by *Glycine max* L.merr, that play significant roles in plant-microbe interactions and pathogen defense. These phenolic compounds have also been implicated in the prevention of such serious health conditions as hormone dependent cancers, osteoporosis, heart disease, menopausal symptoms, and loss of cognitive function. Because of these benefits most of the research surrounding the synthesis and function of isoflavones is targeted to increasing the isoflavone levels in soy products. A prerequisite for selection of high isoflavone producing soybean lines in conventional breeding programs is a simple and efficient selection method. The current project is based on the hypothesis that the expression of genes involved in the production of isoflavones, as determined by miroarray analysis, is significantly correlated with isoflavone content in soybean. We are constructing cDNA arrays representing approximately 30 genes (both structural and regulatory) involved in isoflavone biosynthesis. The arrays will be used to assay for isoflavone gene activity in developing seeds and leaves of 3 soybean varieties previously characterized as being low in all isoflavones, high in the specific isoflavone genistein, or high in the specific isoflavone daidzein. They will also be used to screen a population of recombinant inbred lines segregating for isoflavone content. Isoflavone levels in the tissues will be measured by HPLC and this data will be correlated with gene expression data obtained from cDNA hybridizations to the microarray to test the usefulness of the array to screen for isoflavone content in soybean lines. As a preliminary test, the genes were spotted onto a nylon membrane and probed separately with DIG-labelled cDNA isolated from high and low isoflavone varieties. The hybridization revealed clearly visible differences in expression level between the genes on the membrane.

P9: Characterization of the nodulation phenotype of E151, a pleiotropic pea (*Pisum sativum L.*) mutant

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E151 (*sym15*) is characterized as a pleiotropic pea (*Pisum sativum L.*) mutant. It has been described as having short lateral roots, a short primary root, and a shorter epicotyl than that of the wild type Sparkle. Furthermore, it was ascribed the designation of a low nodulator since nodulation was rare after 4 weeks of growth (Kneen et al., 1994). The goal of our study is to further characterize the nodulation phenotype of this mutant. In order to accomplish this, three approaches were undertaken. Firstly, plants were inoculated with *Rhizobium leguminosarum* biovar *viciae*, were harvested 42 days after inoculation (DAI), and nodules were counted. To our surprise, E151 had numerous pink nodules; E151 had 130 nodules whereas Sparkle formed approximately 330 nodules. Secondly, nodule development was studied. Guinel and Sloetjes (2000) had dissected the process of nodulation into 6 events: (A) infection threads (IT) in root hair or epidermis, (B) IT in cortex and not associated with division, (C) IT in cortex but associated with cortical cell division, (D) nodule primordium, (E) emerging nodule, and (F) mature nodule. For our purpose, plants were inoculated with rhizobia containing a lacZ promoter. The fixed and cleared whole root sections were reacted with the X-Gal substrate, this produced a blue colour allowing the rhizobial path to be visualized. Preliminary data suggest that nodule development in E151 is arrested at stage C. Finally, nodulation control was assessed by performing grafts between Sparkle and E151. This study is preliminary but it will allow us to determine whether nodulation in E151 is shoot or root controlled.

P10: Identification of common bacterial blight resistance genes in *Phaseolus vulgaris*

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Common bacterial blight (CBB) is endemic to all regions of the world where dry beans (*Phaseolus vulgaris*) are grown. This disease presents itself as brown lesions of leaves and pods and is caused by *Xanthomonas axonopodis* pv. Phaseoli. Recently, a CBB-resistant cultivar, OAC-Rex was developed (registration no. 5491). It was tested as OAC 95-4 and was derived from a cross between HR20-728 and MBE 7 made in 1988. MBE 7 was a selection from the cross of ICA Pijao/PI 440795//Ex Rico 23 and was used to provide the CBB resistance. Another CBB-resistant line, HR67, was produced by a series of crosses between Centralia/3/HR13-621//OAC Rico/XAN159 (Yu *et al*. 2000). OAC-Rex represents the first CBB resistant cultivar released in North America, however the genes responsible for this resistance not yet been identified. To simplify the search for genes associated with CBB resistance in these cultivars, binary-bacterial artificial chromosome (BiBAC) libraries were created by ligating high molecular weight DNA from OAC-Rex and HR67 into the BiBAC2 vector. Each of these libraries contain approximately 30,000 clones, representing a library depth greater than 6 for each cultivar. The libraries will be screened with CBB resistance-associated molecular markers identified by previous studies. Twenty-five clones were identified in the initial screen of the OAC-Rex library, and the presence of the PVctt001 marker was confirmed by PCR. Positive clones will be infiltrated into young susceptible bean lines using *Agrobacterium tumafaciens,* and the plants will be infected with *X. axonopodis*. Clones containing genes for CBB resistance are expected to display a significant reduction in size of *X. axonopodis*-induced lesions. The clones providing protection will be subcloned and used for additional rounds of infiltration until a gene-sized fragment is identified.

P11: The effect of storage protein deficiency on protein composition in mature seeds of common bean

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We have used an approach combining two-dimensional gel electrophoresis followed by LC-ESI-MS to identify and compare protein profiles in mature seeds for three genotypes of common bean. We are taking advantage of the availability of genetic stocks combining deficiencies in the major storage proteins, phaseolin (Phs), phytohemagglutinin (Lec) and arcelin (Arl) (Osborn et al. 2003 Crop Sci. 43, 1570). The lines being used, SARC1, SMARC1-PN1 (*phs*) and SMARC1N-PN1 (*phs*, *lec*, *arl*) share a common Sanilac background. Genotypes were initially confirmed by SDS-PAGE. The three lines had a similar seed nitrogen, carbon, and total protein content. However, storage protein deficiency was significantly correlated with higher levels of cytoplasmic proteins. An analysis of free amino acids revealed that most were significantly elevated in response to storage protein deficiency. The amino acids asparagine and arginine were markedly more abundant in the mutant line, by 1.5- and 3.8 fold respectively. An analysis of replicate two-dimensional polyacrylamide gels was done using ImageMaster 2D Platinum 6.0 software. This analysis identified 350 ± 45 spots in SARC1 and 283 ± 43 spots in SMARC1N-PN1. Additionally, 54 spots were unique to SARC1, 32 of which were tentatively assigned to phaseolin and 14 to the lectin superfamily. In the storage protein deficient line, 17 spots appeared as novel proteins. In total, approximately 130 protein spots were common to both SARC1 and SMARC1N-PN1, 40 of which were found to be up-regulated in response to storage protein deficiency. A large fraction of proteins (69%) showed no change in expression in the storage protein deficient line. One-hundred and fifty spots of interest will be excised from the gel and subjected to analysis by LC-ESI-MS. Preliminary results of these studies will be presented.

P12: Cuticular cracks in soybean seed coats: development and effect on permeability

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The seeds of soybean (*Glycine max* [L.] Merr.) are classified as “impermeable” or “permeable”. According to earlier studies, impermeable seeds have intact cuticles, while permeable seeds have microscopic cracks 20 to 200 µm long which allow relatively rapid water entry during imbibition. Although cultivars such as OX736 and H63 are known as “permeable,” a very low percentage of their seeds are impermeable. Conversely, OX951 is known as an “impermeable” cultivar but one quarter of its seeds can be permeable. Seed surfaces of these anomalous seeds were examined for cracks with SEM. The results showed that the trait of permeability was correlated with cuticular cracking and not with cultivar, confirming the earlier conclusion. The time of formation of cuticular cracks during seed development was also investigated for cultivars OX736 and OX951. Most seeds of the latter never formed cracks. Cracks became apparent in the former cultivar just before the seeds reached their maximum size in the pods, suggesting that crack formation may be caused by seed surface expansion.

P13: The role of two *NAC*-like transcription factors during Age-Related Resistance in Arabidopsis

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Age-related resistance (ARR) is a term that describes the relationship between plant development and pathogen resistance. As Arabidopsis matures, it becomes more resistant to normally virulent strains of *Pseudomonas syringae* pv. *tomato* (*Pst*) bacteria. Young plants (3 weeks post germination, wpg) support high levels of *Pst* growth (10^7 colony forming units/leaf disc), while mature Arabidopsis plants (6 wpg) exhibit a significant 10-100 fold

decrease in *Pst* growth. ARR has been correlated with flowering and salicylic acid (SA) accumlation, but SA does not play its normal signaling role in up-regulating *Pathogenesis Related* genes. To identify genes involved in ARR, a reverse genetics/microarray approach was utilized. Gene expression was compared between mock- and *Pst*-inoculated mature plants 12 hours post inoculation (hpi). Two *NAC*-like transcription factor genes were up-regulated in this microarray experiment, suggesting that these *NAC*-like genes may be important for ARR. To determine if these genes are required for ARR, T-DNA insertion mutants were obtained. Young *nac-like 1* and *2* mutants showed similar levels of basal resistance to *Pst* compared to the wildtype, whereas mature *nac-like 1* and *2* mutants were 2- and 7-fold more susceptible to *Pst* compared to the wildtype, respectively. This suggested that the *NAC*-like genes are required for ARR. To determine the expression pattern of the *NAC-like 2* gene during ARR, RT-PCR was performed on wildtype plant samples that were mock- and *Pst*-inoculated. *NAC-like 2* expression was detected 6 and 24 hpi in *Pst*-inoculated samples. This suggests that *NAC-like 2* is expressed as early as 6 hpi during ARR. Our results suggest that these putative *NAC*-like transcription factors play an important role during ARR.

Yeast two-hybrid analyses will be applied to identify the host proteins targeted by each effector. Individual effector genes will also be expressed in strains of *P. syringae* that differ in virulence on *Arabidopsis*. Preliminary analyses indicate that the addition of even one effector can dramatically alter the infection phenotype of these bacterial transformants. Each effector gene will also be introduced into *Arabidopsis* behind an inducible promoter, which will enable microarray analyses of gene expression profiles as a global assessment of the impact of effectors on host cell metabolism and signaling. Plants expressing effector genes will also serve as a valuable resource for affinity purification of effector-host protein complexes as well as additional infection experiments. In parallel with this work, a high-throughput chemical genetic screen will be conducted to identify small molecules that reduce the susceptibility of *Arabidopsis* seedlings to infection by a virulent strain of *P. syringae*. Overall, this investigation draws on a variety of powerful experimental techniques that will not only significantly advance our understanding of the function of specific effector proteins, but also clarify the larger picture of *P. syringae* pathogenesis.

P14: Systems biology of *Pseudomonas syringae* virulence

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Pseudomonas syringae pathogenesis on susceptible host plants depends in large part on the delivery of effector proteins into host cells via the type III secretion system. While effectors appear to promote pathogen virulence by the suppression of basal host defences, their exact functions are generally unknown. Here, we describe a systems approach for elucidating the functions of a number of recently identified effector proteins from *P. syringae* pv. *maculicola* ES4326, a virulent pathogen of *Arabidopsis*.

P15: Methylation in the chloroplast DNA of flax (*Linum usitatissimum*)

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In the total DNA extracted from the cotyledons of seedlings and shoot tips of flax (*Linum usitatissimum*), 10-14% of the cytosines are methylated and the level of methylation in these tissues increases with tissue age and differentiation. Early-flowering flax lines, derived from treatment with 5-azacytidine, have a lower percentage of methylation than their corresponding controls. The study reported here was conducted to determine how much, if any,

of the methylation seen in the total DNA is a result of methylation in the chloroplast DNA, using one of the early-flowering lines and its corresponding control line. It demonstrated that flax chloroplast DNA is methylated, and that, for the tissue used (cotyledons, shoot tips, and total leaves at 21 days of age), the level of methylation in the total DNA was the same in both lines but the level of methylation in the chloroplast DNA was lower for the early flowering line than for the control line. Thus, the plant-line difference for chloroplast DNA mirrored the plant-line difference seen, previously, in total DNA from cotyledons and shoot tips, but the absence of a plant-line difference in the total DNA resembled that seen in total DNA from older tissues. In addition, there were changes in the level of chloroplast methylation, in both flax lines, as a consequence of keeping the plants in the dark prior to chloroplast isolation. The results seem to suggest that the hypomethylation induced during the derivation of the early-flowering line occurred in both nuclear and chloroplast DNA, that the level of methylation is developmentally regulated in both types of DNA, and that the effects of development on methylation level in the two types of DNA are not synchronized.

P16: Kinetic analysis of a recombinant *Arabidopisis* succinate semialdehyde/glyoxylate reductase

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Constitutive expression of an *Arabidopsis thaliana* (L.) Heynh cDNA (GenBank Acc. No. AY044183) in a succinic semialdehyde (SSA) dehydrogenase-deficient yeast (*Saccharomyces cerevisiae*) mutant enables growth on (-aminobutyrate and significantly enhances the accumulation of (-hydroxybutyrate. In this report, the cDNA (designated here as *AtSSA/GR*1) was functionally expressed in *Escherichia coli*, and the recombinant protein purified to homogeneity. Kinetic analysis of substrate specificity revealed that the enzyme catalyzed the conversion of SSA to (-hydroxybutyrate (K_m SSA = 0.87 mM) and glyoxylate to glycolate (K_m glyoxylate = 4.5 μM) via an essentially irreversible, NADPH-based mechanism. The enzyme had a 250-fold higher preference for glyoxylate than SSA based on the performance constants (k_{cat}/K_m), and with the exception of 4-carboxybenzaldehyde, at least a 100-fold higher preference for SSA than all other substrates tested (formaldehyde, acetaldehyde, butyraldehyde, 2-carboxybenzaldehyde, glyoxal, methylglyoxal, phenylglyoxal, phenylglyoxylate). Initial-velocity, dead-end inhibition and product inhibition studies revealed that the kinetic mechanism was ordered Bi Bi, involving the complexation of NADPH to the enzyme before SSA or glyoxylate, and the release of $NADP^+$ before (-hydroxybutyrate or glycolate, and was subject to dramatic inhibition by $NADP^+$. It is suggested that succinic semialdehyde/glyoxylate reductase functions in the detoxification of SSA and glyoxylate that are produced, respectively, during the metabolism of (-aminobutyrate and glycolate in plants.

P17: Transformation of *Acinetobacter* sp. with the *cp4 epsps* gene

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Glyphosate [*N*-(phosphonomethyl)glycine] prevents the biosynthesis of aromatic amino acids by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme present in plants, bacteria and fungi. Roundup Ready® (RR) soybean [*Glycine max* (L.) Merr.] contains *cp4 epsps*, a glyphosate-tolerant

version of the gene. The purpose of the current work is to test for the movement of DNA from transgenic plants to soil microorganisms, using *cp4 epsps* as the tracking gene. *Acinetobacter* sp. is a ubiquitous microorganism in the plant rhizosphere that has been shown to be an opportunistic plant colonizer able to multiply *in planta*. It is naturally competent to take up DNA. The growth of *Acinetobacter* sp. BD413 in LB broth with varying concentrations of glyphosate was investigated in order to develop a screen for *cp4 epsps* gene transfer. Growth of *Acinetobacter* was completely inhibited at 20 mM glyphosate. The *cp4 epsps* gene was amplified from RR soybean DNA using gene-specific primers and inserted into the broad-host range plasmid pRANGER-BTB-3® (pRANGER:EPSPS) downstream of an arabinose-inducible promoter and the construct was electroporated into *Acinetobacter.* A truncated version of the *cp4 epsps* gene was also inserted into the plasmid (pRANGER:ΔEPSPS) and electroporated into *Acinetobacter*. Colony PCR using vector- and insert-specific primers confirmed transformation of *Acinetobacter* with the two vectors. *Acinetobacter* pRANGER:EPSPS and *Acinetobacter* pRANGER:ΔEPSPS were grown in modified M63 medium + arabinose with varying concentrations of glyphosate to determine whether the *cp4 epsps* gene conferred an increase in tolerance to the herbicide.

P18: AtFIN3: A host target of the *Pseudomonas syringae* Type III effector HopF

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The ability of *P. syringae* to cause disease on a broad range of plant species is dependent on type III effector proteins delivered into plant-host cells by the type III secretion system. A few well characterized effectors have been demonstrated to promote pathogen growth, suppress host defenses and elicit host-specific disease symptoms by interacting and modifying targets within the host-cell. However, functional roles and targets of most effectors have not been assigned, and therefore, the mechanisms of bacterial disease induction are not clearly understood. Host targets of *P. syringae* type III effectors likely include proteins that play an integral role in the coordinated defense-responses induced during attempted infection and/or proteins required for nutrient acquisition in order to promote pathogen growth. Yeast two-hybrid analysis has identified eight *Arabidopsis thaliana* proteins that interact with $HopF_{Pto}$ (AtFIN1-8; *A. thaliana* HopF Interactor). Preliminary analysis of homozygous T-DNA insertion lines in the *AtFIN3* gene illustrate that reduced *AtFIN3* expression levels result in increased resistance to *P. syringae* pv. *tomato* DC3000 infection. Furthermore, AtFIN3 mRNA levels detected by RT-PCR rapidly decrease in *Arabidopsis* Col-0 plants infected with *P. syringae*. Differential rates of decrease and restablishment of *AtFIN3* mRNA levels are observed in plants infected with virulent versus non-virulent *P. syringae* strains. These results indicate that AtFIN3 may play a negative regulatory role in plant defence. The AtFIN3 protein is part of a four member family of *Arabidopsis* proteins that contain WD-40 domains which share sequence similarity to components of the proteasome. Interestingly, bioinformatic analyses of *AtFIN3* predict two splice variants; with and without the WD-40 domain. The role of these *AtFIN3* splice variants as well as *AtFIN3* homologs in plant defence is currently being investigated.

P19: Suppression and induction among six members of the defensin gene family in *Nicotiana benthamiana* during compatible and incompatible plant-pathogen interactions

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Plant defensins are small, basic, cysteine-rich proteins with antimicrobial activity. In most plants, they are encoded by small multigene families and have been shown to have variable expression in different plant tissues. Four members of the family were identified in *Nicotiana benthamiana* by searching of the *N.*

benthamiana Gene Index, Release 2.0 (http://www.tigr.org) and two other genes were identified by comparison to highly conserved defensins among other Solanaceous plants. *NbDef1.1*, *NbDef1.2*, *NbDef1.3* and *NbDef1.4* are short defensin of about 78 amino acids, while *Nbdef2.1* and *NbDef2.2* are larger defensins of about 105 amino acids with an additional 27 to 33 acidic amino acid C-terminal domain. To study the expression of these genes, relative RT-PCR was used with a translation elongation factor-1α gene as an internal control. *NbDef1.1*, *NbDef1.2*, *NbDef1.3* were expressed in healthy leaves, flowers, developing seeds, roots and stems. *NbDef1.4* was only expressed in developing seeds and flowers, whereas *Nbdef2.1* and *NbDef2.2* were only expressed in flowers. In a compatible interaction of *N. benthamiana* leaves with *Pseudomonas syringae pv. tabaci*, expression of *NbDef1.1* and *Nbdef1.3* were similarly down-regulated, while expression of *Nbdef1.2* and *Nbdef2.1* were significantly up-regulated. In an incompatible interaction of *N. benthamiana* expressing *Pto* with *P. syringae pv. tabaci* expressing *AvrPto*, down-regulation was only observed for *NbDef2.1*, which corresponded with the timing of the hypersensitive response. In contrast, none of the six defensin genes showed changes in compatible interactions with *Colletotrichum destructivum* and *Colletotrichum orbiculare*, the causal agents of anthracnose.

P20: Expression of the porcine β-defensin-1 (*pbd-1*) gene in plants

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Defensins are a class of antimicrobial peptides that are part of the defense mechanisms of a broad range of organisms, including mammals, arthropods and plants. All defensins are cationic, cysteine-rich, and relatively small in size. Defensins may provide an alternative to antibiotics for animals and a means for increasing disease resistance in plants. For these reasons, *Nicotiana tabacum* plants were transformed with the porcine β-defensin-1 (*pbd-1*) gene using two approaches to overcome the typically low levels of accumulation of small charged peptides in transformed plants. A codon modified *pbd-1* gene was fused with a fragment of a plant gene, *po2*, which contains the 5' UTR, the signal sequence, and a portion of the negatively charged domain of the mature PO2 peptide. It was hypothesized that this fusion with a plant protein would protect PBD-1 protein from degradation. For comparison, the codon-modified *pbd-1* was fused with the 5' end portion of the native pig *pbd-1* sequence, which codes for the porcine defensin signal sequence and prosequence. Expression of the defensin transgenes was detected at the RNA level by RT-PCR in T_0 transgenic plants, which were tested for resistance to *Pseudomonas syringae* pv. *tabaci*. Based on the severity of disease symptoms, the transgenic plants appeared to have enhanced disease resistance. However, there were no significant differences in the degree of resistance to *P. syringae* pv. *tabaci* between transgenic plants containing the two different fusion constructs of *pbd-1*. Expression levels may be very low or degradation may occur as defensin proteins were not detected in any transgenic plant by SDS-PAGE and Western analysis. T_2 homozygous plants have been generated and will be tested for disease resistance against different pathogens. Also, different protein extraction and detection techniques will be examined to try to detect and purify the porcine defensin peptide.

P21: Identification of host targets of *Pseudomonas syringae* HopZ cysteine proteases

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The plant pathogen *Pseudomonas syringae* uses the type III secretion system to secrete and translocate effector proteins into the plant. The direct or indirect recognition of these effectors by plant resistance proteins (R genes) induces a defense response, which includes a programmed cell death reaction called the hypersensitive response. In the absence of recognition of the bacterial effector by the plant, effectors contribute to the ability of the pathogen

infect and cause disease. A common class of effector proteins [DSG1] are the cysteine proteases of the HopZ family, which includes HopZ1a (HopPsyH), HopZ1b, HopZ1c (HopPmaD), which are all closely related allelic variants, and HopZ2 (avrPpiG) and HopZ3 (HopPsyV), which are each quite similar to homologous effectors carried by other plant pathogenic bacteria. Ma et al (2006) recently determined that HopZ1a is most similar to the ancestral *P. syringae* allele, and that this family diversified within the *P. syringae* species complex both by the mutational generation of allelic variants, and also by the acquisition of homologs from ecologically similar bacteria via horizontal gene transfer. They further showed that this diversification was driven by the host defense response.

We are interested in identifying host targets of the five HopZ members. We will identify directly interacting partners using the yeast two-hybrid screen. As well, we are constructing transgenic *Arabidopsis* plants expressing the effector proteins to identify effector-host protein complexes and to examine effects on global gene expression. We are also introducing the HopZ members into various strains of *Pseudomonas syringae* to identify virulence or avirulence functions on various host plants. This work will address how differences in host target specificity of one type III effector family contribute to the host range of a particular pathovar of *P. syringae*.

P22: Protein-protein interactions between maize starch synthases and branching enzymes expressed in *E. coli*

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Starch provides the major caloric source for the human population and is also an important industrial commodity. Although the pathway of starch biosynthesis is not completely understood, it is known to involve at least four groups of committed enzymes: ADP glucose pyrophosphorylase is responsible for synthesizing the precursor of starch biosynthesis (ADPglucose), starch synthases (SS) add the glucose moiety from ADPglucose to a pre-existing glucan chain, thus elongating glucan chains, starch branching enzymes (SBE) create branches within amylopectin, and debranching enzymes are involved in trimming the growing glucan chains to allow the semicrystalline structure to form. Previous work has shown the formation of protein complexes between key enzymes of starch biosynthesis using gel filtration chromatography, co-immunoprecipitation and cross-linking experiments. It is proposed that these protein complexes play an important role in the formation of the starch granule. Currently, active recombinant forms of maize SSI, SBEI, SBEIIa and SBEIIb have been overexpressed in *E.coli* in order to understand the process of protein complex formation. A number of experiments have demonstrated the *in vitro* reconstitution of protein complexes previously observed *in planta*. Recombinant proteins have also been attached to affinity chromatography media and used as 'bait' to identify other interacting proteins using maize amyloplast preparations and endosperm extracts.

P23: Dual localization of diacylglycerol acyltransferase 1, an enzyme involved in the biosynthesis of triacylglycerol, to ER and chloroplasts

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Diacylglycerol acyltransferase 1 (DGAT1) is an enzyme that catalyzes the final reaction in the Kennedy, or oil producing, pathway. This reaction typically involves the transfer of an acyl group from acyl:CoA to diacylglycerol to produce triacylglycerol (TAG). DGAT1 is found in a diverse collection of organisms, including humans, mice, and plants, and previous studies have shown it to be localized to the endoplasmic

reticulum of various tissues. In this study, we present confocal microscopy and immunoblot data suggesting that in Arabidopsis embryos and leaves DGAT1 is also located in chloroplasts, which represent another TAG-accumulating compartment of plant cells; TAG is localized to the plastoglobuli of chloroplasts. In addition, DGAT1 from *Brassica napus* (BnDGAT1) has been radiolabelled and tested using in vitro chloroplast import assays. Preliminary assays indicate that this protein has a transit peptide that is removed upon import into chloroplasts; these assays will be repeated using DGAT1 from *Arabidopsis thaliana*. Future assays will be aimed at determining the sub-chloroplast compartment to which DGAT1 is targeted. Interestingly, acyl:CoA is not found in chloroplasts, meaning that the mechanism of TAG biosynthesis by DGAT1 must be different in chloroplasts. It is hypothesized that DGAT1 is involved in using the fatty acids released from thylakoid lipids by the action of lipases into plastoglobuli in the form of TAG, a process that is particularly active during senescence and times of stress.

P24: Expression of therapeutic proteins in tobacco BY-2 cell suspension culture

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In recent studies, many recombinant proteins have been expressed in plants for the production of therapeutic pharmaceuticals. The levels of accumulation of these proteins in their respective plant systems are variable. The human interleukin-10 protein (IL-10) is a homodimeric protein that possesses anti-inflammatory properties and is a potential treatment for inflammatory bowel disease. IL-10 is a good representative of other therapeutic pharmaceuticals, but it does not accumulate to a desirable level in tobacco leaves. We have previously shown that IL-10 targeted to the ER accumulated to levels of 50 ng/mg of total soluble protein (TSP). The same construct produced 250 ng of IL-10 per mg of TSP when expressed in tobacco BY-2 cells. The use of transgenic BY-2 cell lines for the stable *in vivo* production of IL-10 thus provides a viable model for the expression and suitable accumulation of plant-made pharmaceutical proteins. In an effort to simplify purification and reduce its cost, we created constructs that would secrete IL-10 to the BY-2 culture medium. Preliminary results showed levels of IL-10 in transgenic BY-2 calli ranging from 0.05 to 0.15 ng/mg TSP. These calli will be used to produce stable, perpetual cell lines for the examination of accumulation levels, sub-cellular localization, the rate of protein turnover, and optimization of growth conditions of BY-2 cell lines for the economical purification of this therapeutic protein.

P25: The *Arabidopsis thaliana* 36 kDa peroxisomal membrane protein contains two distinct molecular targeting signals

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Although several studies to date have examined the molecular mechanisms responsible for the targeting of peroxisomal membrane proteins (PMPs), a consensus has yet to emerge as to what constitutes a prototypic membrane peroxisomal targeting signal (mPTS). In addition, there is only a limited amount of information on the sorting of plant PMPs since the majority of research on PMP biogenesis has been carried out using yeast and mammalian model systems.

The purpose of this study was to investigate the molecular targeting information within the 36 kDa *Arabidopsis thaliana* PMP (PMP36), a member of the ATP/ADP-binding cassette family proposed to be responsible for transporting of small soluble molecules across the peroxisomal boundary membrane. Using site-directed mutagenesis and immunofluorescence microscopy we employed plant suspension-cultured cells as an *in vivo* experimental system to demonstrate that PMP36 contains two distinct, non-overlapping mPTSs. One targeting signal consists of a majority of the protein including five (of six) transmembrane domains (TMDs) and their adjacent N-terminal soluble regions. The second targeting signal is a relatively shorter region of the protein consisting of the C terminus including the sixth TMD. Notably, neither of these two mPTSs within PMP36 contain any obvious similarities in their primary amino acid sequences, suggesting that they mediate peroxisomal sorting by different targeting mechanisms. In addition, these data imply that the two PMP36 mPTSs are functionally distinct and thus may serve unique roles in targeting to the wide range of peroxisomes classes (e.g., seed glyoxysomes, leaf and root peroxisomes, gerontosomes, etc.) that exist during plant growth and development. This latter premise, as well as results from mutational analyses aimed at characterizing the precise nature of the two mPTSs within PMP36 and how they compare with the targeting information defined previously for other PMPs in various organisms will be presented.

P26: Using two putative *AROGENATE DEHYDRATASEs* (*ADT*s) from *Arabidopsis* to complement the *pha2* phenotype in yeast

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Phenylalanine (PHE) is an aromatic amino acid that is essential for protein synthesis in all living organisms, but is only synthesized by microbes, fungi and plants. In plants PHE is also a precursor for a wide range of secondary metabolites (such as flavonoids and lignins) which contribute to a plant's survival as they provide structural support and protection.
PHE biosynthesis has been described to occur in one of two ways, either using the prephenate or the arogenate pathway. In the prephenate pathway, prephenate is converted to PHE through a phenylpyruvate intermediate where a prephenate dehydratase (PDT) catalyzes a decarboxylation and dehydration reaction to form phenylpyruvate. In the arogenate pathway, PHE is synthesized from prephenate via arogenate and an arogenate dehydratase (ADT) catalyzes the decarboxylation and dehydration reaction to convert arogenate to PHE. It has been suggested that plants predominantly use the arogenate pathway as ADT activities have been biochemical detected in plant cell extracts. A family of six putative *ADT*s has been identified in the *Arabidopsis* genome, however, the biochemical activity of the corresponding proteins has not yet been determined. In contrast, the yeast *Saccharomyces cerevisiae* genome encodes a single *PHA2* gene encoding a PDT. For this study, two *ADT* genes were cloned into the yeast pYES2/NT expression vector as part of a *GAL1* regulated expression cassette. The *ADTs* were either cloned as full-length sequences or *ADT* sequences that lack a putative signal peptide sequence for organelle targeting. Vectors containing were transformed into a *pha2* knockout yeast strain that is unable to synthesize the endogenous PDT and is unable to grow unless PHE is supplied in the growth medium. We report the ability of *Arabidopsis ADT*s to complement the *pha2*phenotype, allowing the yeast mutant strain to grow in absence of PHE.

P27: Analysis of *AROGENATE DEHYDRATASE* (*ADT*) expression patterns in *Arabidopsis thaliana* using RT-PCR

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In *Arabidopsis thaliana* a family of six genes has been identified as they share a high degree of sequence similarity to bacterial *PREPHENATE DEHYDRATASE*s (*PDT*s). PDTs convert prephenate to phenylpyruvate as part of the prephenate pathway for phenylalanine (PHE) biosynthesis. However, it has also been reported that PHE can be synthesized via the arogenate pathway, which requires the functionally similar enzyme, AROGENATE DEHYDRATASE (ADT). Analogous to PDTs, ADTs catalyze a decarboxylation/dehydration step using arogenate as a substrate to form PHE. Biochemical evidence suggests that higher

plants synthesize PHE through the arogenate pathway indicating the six *Arabidopsis* genes likely encode ADTs.
In plants PHE is required for protein synthesis as well as the synthesis of many secondary metabolites (such as lignin, flavonoids, melanins, phenols and tannins). Furthermore, it has been reported that PHE biosynthesis is regulated in response to cell internal and environmental cues including a number of stress responses including cold, heat and drought. However, it is unclear why *Arabidopsis* needs six *ADT* genes to fulfill its requirements for PHE synthesis, if all six genes play equal roles or if all genes contribute equally to various stress responses. We predict that the diverse needs for PHE are mediated through individual *ADT*s and one can therefore anticipate that the expression patterns for the *ADTs* will be distinct. To test this hypothesis, we have initiated the analysis of mRNA expression patterns of three *ADT*s using a modified reverse transcriptase (RT)-PCR protocol to study the expression of individual *ADTs* in various tissues of mature *Arabidopsis* plants raised under standard growth conditions. RT-PCR determined that *ADT1*, *ADT2*, and *ADT3* are expressed in all tissues examined. *Arabidopsis thaliana* encoding proteins with a similar domain structure: a putative N-terminal signal peptide, an internal catalytic ADT domain and a C-terminal ACT domain, a putative ligand binding domain for post-translational feedback regulation. *In silico* analyses indicate that the putative signal peptides direct at least four of the six ADTs to the chloroplast. Alternative predicted locations are mitochondria or the cytosol. PHE biosynthesis is hypothesized to occur in chloroplasts or the cytosol depending on the use of PHE. We have prepared ADT-GFP fusion constructs for ADT1 and ADT3 to determine their subcellular localization. Both genes were cloned as full-length *ADT* sequences or as *ADT*s lacking the N-terminal putative signal peptide. Preliminary transient expression studies in onion epithelial cells show specific subcellular localization of the full-length ADTs into organelles while proteins lacking the signal peptide are found to have a much more diffused pattern.

P28: Transient expression of *Arabidopsis* ADT1 and ADT3 as GFP fusions in onion epithelial cells

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Phenylalanine (PHE) is one of three essential aromatic amino acids and only bacteria, fungi and plants are able to synthesize PHE *de novo*. It serves as a precursor for protein biosynthesis in all organisms. In plants PHE is also required for the synthesis of many secondary metabolites including lignin, flavonoids, melanins, phenols and tannins. In plants, it has been suggested that the arogenate pathway is used for PHE synthesis relying on the enzymes prephenate aminotransferase (PAT) and arogenate dehydratase (ADT) to convert prephenate to arogenate and phenylalanine, respectively. Six putative *ADTs* have been identified in

P29: Is there a role for ESCRT in the formation of multivesicular peroxisomes during tombusvirus infection?

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Peroxisomes are highly dynamic organelles in terms of their shapes, mobility and metabolic functions. Peroxisomes are also important sites for the development of some viral pathogens. Specifically, certain tombusviruses recruit peroxisomes for viral RNA replication and in doing so cause an extensive inward vesiculation of the organelle's boundary membrane leading to the formation of a peroxisomal multivesicular body (pMVB). While it is unclear how the internal

vesicles of a pMVB are formed, results from previous studies suggest that the two viral components of the tombusvirus replication complex, namely a 33 kDa RNA-binding protein (p33) and its translation read-through product, a 92 kDa RNA polymerase (p92), are required for their biogenesis. Here we extend this proposed role for p33 and p92 in pMVB formation by providing evidence that, similar to retrovirus Gag proteins such as those from Ebola and HIV, p33 and p92 recruit constituents of ESCRT (Endosomal Sorting Complex Required for Transport) to facilitate internal vesicle formation at the peroxisomal membrane. First, the formation of internal vesicles in pMVBs is topologically equivalent to the ESCRT-dependent formation of internal vesicles within late endosomes and the release of retroviral particles from the plasma membrane in infected mammalian cells. Second, both p33 and p92 possess sequences that resemble the late-budding domain in the Ebola VP40 Gag protein, suggesting that analogous to VP40, p33/p92 mimic the activity of early ESCRT components to recruit the remaining ESCRT machinery to the peroxisomal membrane. Third, yeast two-hybrid analyses revealed that C-terminal soluble portions of p33 and/or p92, including their putative late-budding domain, interact specifically with the Arabidopsis ESCRT components Vps23 and Vps28. Finally, transient expression of Vps23 or Vps28 in TBSV-(co)transformed tobacco cells revealed that both ESCRT components relocalize to pMVBs, suggesting that these and possibly other components of the ESCRT machinery are involved in pMVB biogenesis.

P30: "Caterpillar-specific" transcriptional responses in the model legume, *Medicago truncatula*

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Induced responses to caterpillar herbivory have traditionally been viewed as an extension of the general plant response to mechanical damage; however, it is now recognized that although there are shared aspects, insect herbivores elicit distinct responses in plants. Caterpillar-specific responses are thought to be mediated by elicitors, such as volicitin and glucose oxidase, present in the oral secretions of actively-feeding larvae. This study investigated early transcriptional responses of the model legume, *Medicago truncatula* L., to herbivory by caterpillars of the beet armyworm, *Spodoptera exigua*. Differentially expressed genes were identified by the differential display technique, cDNA-amplified fragment length polymorphism (cDNA-AFLP). To identify general wound induced responses, a subset of plants were mechanically wounded in an attempt to mimic caterpillar herbivory. To identify specific plant responses to elicitors present in the caterpillar saliva, other plant groups were subject either to herbivory by caterpillars with normal salivary secretions or to herbivory by caterpillars that had their spinneret, the appendage through which labial saliva is secreted, burnt shut. Twenty gene fragments have been identified and sequenced which represent 17 putative differentially expressed genes. General transcript expression patterns ranged from i) wound-specific ii) wound- and caterpillar-specific and iii) caterpillar-specific. These preliminary results suggest that plants are able to differentiate between caterpillar herbivory and mechanical damage and that transcriptional response are initiated within one hour after caterpillar infestation. Future research will validate differentially expressed genes by Q-RT-PCR and will address the specific influence of oral elicitors on the early response of *M. truncatula* to wounding and caterpillar herbivory.

P31: Characterization of transgenic potato roots transformed to underexpress cytosolic triose phosphate isomerase

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Triose phosphate isomerase (TPI) catalyzes the interconversion of dihydroxyacetone–P and glyceraldehyde 3-P in the glycolytic pathway. This reaction is at equilibrium and TPI is generally not considered as a regulatory enzyme in plant metabolism. We have previously shown that the cytosolic isoform of the enzyme constitutes the bulk of extractable TPI activity and is developmentally regulated in potato. We have used a transgenic approach to examine the

metabolic function of cytosolic TPI. For this, an antisense construct for cytosolic TPI was introduced in potato using the binary vector pGA643 and *Agrobacterium rhizogenes*. We obtained a population of transgenic root clones displaying 30-100% of TPI activity found in control clones carrying an empty binary vector. Antisense and control roots were subjected to Western blot analysis and analytical chromatography on DEAE fractogel. The data show that the antisense strategy only affects the cytosolic TPI isoform, while levels of plastidic TPI activity remain constant. Transgenic roots were characterized with respect to the activity of glycolytic enzymes, their metabolite contents and various carbon fluxes. Metabolite profiling of sugars, organic acids and amino acids as well as ^{14}C-glucose flux analyses indicate that an important reduction of cytosolic TPI activity affects several aspects of root carbon metabolism. Antisense roots containing the lowest amounts of cytosolic TPI show an increased flux of C through the pentose phosphate pathway. Consistent with these findings, these clones also have increased levels of aromatic amino acids and an increased capacity for lipid synthesis. These results reveal that cytosolic TPI plays a role in the balance of carbon fluxes in plant root metabolism.

P32: Cloning, purification and characterization of a cytosolic peroxiredoxin from *Solanum chacoense*

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Peroxiredoxins (Prx) are a novel family of redox enzymes involved in the reduction of H_2O_2 in the different cellular compartments. A full-length Prx (ScPrx1) was cloned from *Solanum chacoense*, a wild relative of the cultivated potato. Analysis of the predicted primary sequence suggested that the 162 amino acids long protein product is a cytosolic Prx isoform. It was expressed as a (6xHis)-tagged protein in *E. coli*. The recombinant enzyme was active and could be purified to electrophoretic homogeneity in a single step using a metal affinity chromatography column. The purified protein was characterized using an end-point colorimetric assay. (6xHis)-ScPrx1 reaction was linear with respect to time and amount of enzyme in the assay. The enzyme displayed typical Michaelis-Menten kinetics with respect to its substrate H_2O_2 and exhibited an unusual lack of sensitivity to pH. (6xHis)ScPrx1 was also shown to be active in the protection of DNA from oxidative damage.

P33: Partial purification of the most abundant hexokinase isoform from potato (*Solanum tuberosum*) tubers

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Hexokinase catalyzes the phosphorylation of hexoses to generate the corresponding hexose phosphate. Recent findings from our laboratory suggest that, in plants, hexokinase exerts a high degree of control on the phosphorylation of glucose to glucose 6-phosphate. It could therefore play an important role in the control of hexose entry in the glycolytic and the pentose phosphate pathways. In addition to this catalytic function, hexokinases also act as hexose sensors. However, despite its importance, this enzyme has never been purified to homogeneity from a plant tissue. The facts that hexokinase

exists as multiple isoforms and is generally present in very small amounts have been the main problems that have held up progress in that area. The aim of this project is to purify and characterise hexokinase from potato tubers. In this tissue, we have found that at least 4 hexokinase isoforms can be separated by hydrophobic interaction chromatography on Butyl Sepharose. The most abundant isoform was subjected to further purification. Using a series of chromatographic separations on Cibacron Blue dye, DEAE Fractogel, hydroxyhapatite, Phenyl Superose and Mono-Q we were able to partially purify this isoform . The final fraction of the purification was analyzed by SDS/PAGE. Although it was not homogeneous, it contained a major protein band around 50 kDa. This band cross-reacted with affinity purified anti-(*Solanum chacoense* recombinant hexokinase) IgGs. The hexokinase specific activity after hydroxyapatite column was 15.3 U/mg protein, with a purification-fold of 3955, and a yield of 7.19% that corresponds to the highest value ever reported from a plant source. Future efforts will focus on completing the purification and the characterization of the kinetic properties of this isoform.

P34: Failed root growth in common bean (*Phaseolus vulgaris* L.) dwarf plants studied under different temperature regimes, externally supplied sucrose and $^{14}CO_2$

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Crosses between certain bean genotypes result in a characteristic dwarfing of F_1 plants, which reduces growth sufficiently to prevent the production of F_2 seed. This dwarfing results from a dosage dependent effect of alleles at two complementary loci, DL_1 and DL_2. When a plant is heterozygous at both loci ($DL_1dl_1DL_2dl_2$) growth is severely reduced, and when a plant is homozygous ($DL_1DL_1DL_2DL_2$) lethal dwarfing occurs with failed root growth known as "F" plants. Grafting studies demonstrated that DL_1 acts in the root system and DL_2 in the shoot. Root growth can be achieved in both "F" plants and lethal graft combinations by adding sucrose to the growth medium. A series of $^{14}CO_2$ experiments were conducted on self-grafted parents and lethal graft combinations. These grafted plants were also grown at different temperatures to further our understanding of the nature and causes of 'lethal dwarfing' in common beans.

Normal self grafted Ca/Ca and lethal grafted Pd/Ca plants grown on MS media with and without sucrose were fed with $^{14}CO_2$ and 24 hours later the allocation of ^{14}C was determined. In normal Ca/Ca and Pd/Pd plants growing without exogenous sucrose, 4 and 10%, respectively, of ^{14}C was allocated to roots and 2 and 8%, respectively, to the stem below the graft union. In the lethal graft Pd/Ca, photosynthesis was drastically reduced and 10% of ^{14}C was allocated to the stem below the graft union and less than 2% of ^{14}C was recovered from the roots. For all the graft combinations when sucrose was added to the media, very little ^{14}C was recovered below the graft union and the roots. Different growth parameters were studied on grafted plants under three different temperatures conditions. Root growth of the lethal combination (Pd/Ca) was inhibited under all temperature conditions. The data indicate that the physiological and biochemical basis of the lethal dwarfism may be the inability of the roots to receive endogenous assimilates.

P35: Investigating the role of the Actin cytoskeleton in maintaining cell-cell contact during plant development

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Cell-cell connectivity forms the basis for the supra-organismal nature of higher plants. Each plant cell is complete in itself as far as its self-sustaining capability is concerned. However, millions of plant cells are required to create a complete plant that is capable of coordinated growth and development as a single organismal unit. Every developmental response displayed by a plant, from its germination, to resource allocation and myriad defense mechanisms and to its ultimate death relies on cell-cell communication. Whereas plasmodesmata-aided symplastic connectivity as well as apoplastic

communication channels are well known in plants, the role of the cytoskeleton in promoting cell-cell adhesion and communication is still not well understood. Recently, an actin polymerization-mediating complex, the ARP 2/3 complex, has been described in higher plants (Mathur, 2005). The complex comprises of seven subunits, mutations in four of which have been shown to result in aberrantly expanded epidermal cells. Rapidly elongating epidermal cells of the hypocotyl and petiole in the *Arabidopsis* mutants *crooked* (ARPC5), *distorted1* (ARP3), *distorted2* (ARPC2), and *wurm* (ARP2) lose contact with each other and curl out of the axial plane. We are using these mutant cells to understand the role of the actin cytoskeleton in maintaining cell-cell connectivity during coordinated plant morphogenesis. Three different Green Fluorescent Protein (GFP) based probes; GFP-mTalin (labels F-actin); FYVE-GFP (labels endosomes) and GFP-PM (labels the plasma membrane) have been introduced into wild-type and mutant backgrounds and are being subjected to rigorous video imaging and Confocal Laser Scanning Microscopy for extracting information on sub-cellular dynamics. The study is supplemented by high-resolution Transmission Electron Microscopy to provide ultra-structural details of normal and mutant cells during their development.

P36: Dissecting subcellular responses to ROS using simultaneous multicolour live imaging in *Arabidopsis thaliana*

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Plant competitiveness within an ecological niche is intimately linked to its stress management capabilities. A clear understanding of early subcellular response to stress is therefore a prerequisite for devising effective strategies for stress amelioration in plants. Reactive oxygen species (ROS) constitute one of the major instigators of sub-cellular stress response cascades. ROS stimulated responses are rapid, and can vary between transient alterations in the state and behavior of organelles to an irreversible progressive breakdown of the cellular machinery. Sub-cellular ROS generation in plants is easily achieved under laboratory conditions through increased irradiation with ultraviolet rays, manipulation of ambient O2/CO2 ratio, treatment with hydrogen peroxide, or through exogenous application of certain biocides. We have adopted a live imaging approach for **E**arly **I**ntracellular **R**esponse **P**rofiling of **P**lants. The **EIRPP** program is based on the availability of a large number of transgenic *Arabidopsis thaliana* lines carrying multi-colored fluorescent proteins targeted to different subcellular components and compartments (Sinclair et al. 2006). Under this program we have generated judicious combinations of organelle-targeted probes to achieve simultaneous, multi-colour, 4D-visualization of mitochondria, peroxisomes, chloroplasts and cytoskeletal elements and document their response to altered levels of cellular ROS. Our recent focus has been on the response of peroxisomes to oxidative stress. *Arabidopsis* plants carrying a YFP-PTS1 (Mathur et al. 2002) were analyzed for 'normal' peroxisome morphology and motility parameters before being exposed to UV illumination (320 - 400 nm) or 3% H2O2. Under both treatments peroxisome motility decreased and the usual 1.0-1.5 µm long peroxisomes were found to rapidly extend up to 5-10 times their original length. Changes in peroxisome motility and length have been associated with actin dynamics (Mathur et al. 2002), and altered activity of peroxin genes (Lingard and Trelease, 2006) respectively.

P37: Stability of starch biosynthetic enzyme complexes in maize endosperm

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Starch is the major carbohydrate storage product in plants and a major dietary source of energy. Although the core pathway of starch biosynthesis is known, important details regarding the regulation and coordination of the pathway remain unclear. Previous work suggests that formation of protein complexes between enzymes of starch biosynthesis is important in starch granule assembly. In order to understand the role of protein-protein interaction between enzymes of starch biosynthesis in the formation of starch, a number of key enzymes from maize have been overexpressed and used in a variety of experiments to identify and characterize interactions between enzymes of

starch biosynthesis. Starch branching enzyme (SBEIIa) was overexpressed using the Bac-to-Bac Baculo virus expression system. Using the recombinant proteins, pull-down and co-immunoprecipitation experiments were performed to identify proteins interacting with SBEIIa. Results indicate that starch synthase I (SSI), starch synthase II (SSII), and starch branching enzyme I (SBEI) form stable interactions with SBEIIa. Using overexpressed SBEIIa as "bait", stability of complexes at different salt concentrations was verified using pull-down experiments. The SBEIIa / SSI complex dissociated at a NaCl concentration of 0.3 M. However, interactions between SBEIIa and SSII or SBEI appeared to be stable salt concentrations as high as 1M.

P38: Grape *CBF* genes increase stress tolerance and change phenotype in *Arabidopsis*

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The CBF/DREB1 transcription factors regulate an important pathway for increased freezing and drought tolerance in plants. Constitutive expression of the *CBF/DREB1* genes in transgenic *Arabidopsis* plants induces expression from CRT/DRE containing genes and results in an increase in freezing and drought tolerance without prior stimulus. We isolated four *CBF*-like genes, *CBF 1-4*, from both freezing-tolerant wild grape (*Vitis riparia,* Vr) and freezing-sensitive cultivated grape (*Vitis vinifera,* Vv). The grape *CBF* genes are predicted to belong to two different CBF types based on their sequences, and cold-induced expression profile (1,2). Transient expression by agroinfiltration of tobacco leaves showed that the presence of CRT elements in the promoter of a gene is sufficient to induce its expression by VrCBF1 or VrCBF4. Here, we report the function of grape *CBF* genes in homozygous VrCBF1- and VrCBF4-transgenic *Arabidopsis* lines. Overexpression of grape CBFs in *Arabidopsis* increases cold, freezing and drought tolerance and expression of so called *COR* (cold-regulated) genes (*RD29A* and *COR15a*). Moreover, we show that the transgenic *Arabidopsis* lines displayed a prostrate growth habit, late flowering, and thick or double palisade layers in leaves, phenotypes that have been associated with cold acclimation and increased freezing tolerance.

P39: Impact of sex, reproductive status and soil conditions on the mycorrhizal status of wild strawberry plants (*Fragaria virginiana*).

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Vesicular-arbuscular mycorrhizae (VAM) represent a common symbiotic association between plant roots and soil fungi. It is known that a variety of factors can impact the density of fungal association. For example, it is generally noted that mineral deficient soils can increase the fungal structure concentration, but many other parameters have not been examined. In the current study, the VAM density of wild strawberry plants (*Fragaria virginiana*) was assessed over a 9-week period. *F. virginiana* is a gynodioecious species, therefore existing as either a female or a hermaphrodite. The plants were wild populations found at five locations around the Pymatuning State Park (Jamestown, PA). Soil conditions ranged from a fertile, clay

soil to acidic, high-metal soils of abandoned railway lines. Plants were sampled from mid-May to mid-July, spanning the flowering, fruiting and post-fruiting portions of *F. virginiana* reproduction. To directly assess the fungal density in the mycorrhizal association, second and third order roots were cleared and stained with Trypan blue. The quantity of VAM structures (primarily vesicles and hyphae) was then scored using a microscope. Additionally, soil from around the root system was screened to determine the spore density, which served as a rough indicator of fungal reproduction. Changes in fungal density did not produce any strong patterns, though it was noticed that the harsher soils showed a decrease as the season progressed, while the plants growing on more amenable soils showed a greater fungal density. This, and other patterns, will be further discussed.

P40: Response of Thellungiella to water deficits

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Thellungiella salsuginea is an extremophile crucifer that can withstand high salinity, freezing temperatures, and prolonged periods of drought. In Canada, *Thellungiella"*s natural range extends to the Yukon Territories where sub-arctic temperatures and semi-arid conditions prevail. In China, *Thellungiella* has been found on the temperate and saline coast of northeastern China. Our study compares plants from populations originating near Whitehorse, YT and Shandong Province with respect to their drought response and their capacity to recover from water deficits. Plants of the Yukon and Shandong ecotypes were exposed to simulated drought in controlled environment chambers by withholding water. Under growth conditions used, both Yukon and Shandong plants undergo wilting at a RWC of 60%. Normally, the onset of wilting took 7 days for plants that had not previously experienced wilting. Upon a second episode of simulated drought the two ecotypes behaved differently with Yukon plants wilting in 9 days while Shandong plants wilted in 6 days. Thus the drought tolerance of Yukon plants but not Shandong plants improved upon exposure to a single episode of water deficit. Yukon plants also established turgor at a lower solute potential relative to Shandong plants. Gas chromatography-mass spectrometry (GC/MS) is being used to identify organic solutes accumulated in tissues of well-watered and drought stressed plants for both ecotypes. Of over 300 components detected in each chromatogram, a subset of 30 undergo significant drought-responsive changes and among these, three metabolites are present at elevated levels in leaf extracts of plants harvested at a Yukon field site during a dry growing season relative to a year with ample precipitation. These three metabolites show no drought-responsive behaviour in Shandong plants leading to the hypothesis that these metabolites are associated with the superior drought tolerance of the Yukon ecotype.

P41: Involvement of the *Solanum chacoense* MAPKKK ScFRK1 in embryo sac development

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The *Solanum chacoense* Fertilization-Related Kinase 1 (ScFRK1) is a member of the MEKK subfamily of plant MAPKKK that is specifically expressed in ovules. RNA gel blot analysis showed that *ScFRK1* mRNA levels decrease rapidly following pollination. In situ hybridization analysis showed that *ScFRK1* mRNAs accumulate during early ovule development predominantly at the distal end (tip) of the growing integument in developing ovules. Later, *ScFRK1* expression is almost exclusively detected in the egg apparatus cells of the embryo sac. These results suggest both pre- and post-fertilization roles of ScFRK1 in ovule development To assess this, transgenic plants down-regulated in *ScFRK1* expression were generated. No abnormal phenotypes could be observed except for the production of seedless

fruits upon pollination. To define where and when during ovule development abnormalities appeared, cleared ovules from affected transgenic lines were observed by differential interference contrast. At anthesis, higher frequency of abnormal embryo sacs correlated with lower *ScFRK1* expression in these transgenic lines, confirming the link between *ScFRK1* expression and the formation of a functional embryo sac. Using flower buds at various developmental stages it was possible to show that in mutant plants weakly expressing the *ScFRK1* gene, formation of the female gametophyte did not progress further than the functional megaspore stage. No further divisions were observed, leading to the formation of ovules devoid of an embryo sac, explaining the seedless phenotype. Thus pollination led to the production of parthenocarpic fruits, although smaller in size. Using a semi *in vivo* pollen tube growth system *ScFRK1* mutant lines were also shown to be severely affected in pollen tube guidance, confirming the involvement of the egg apparatus cells (synergids and/or egg cell) as the source of attracting molecules.

P42: Tiller senescence level determines the appearance of later leaves in rice.

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Developing cropping calendars to accommodate newly introduced crops requires that the members of the existing crop rotation have flexibility in planting dates. In the midhills of Nepal, changing planting dates exposes the rice crop to new thermal environments changing both the overall duration and the speed of crop establishment. Crop establishment depends on the speed of leaf appearance and the longevity of individual leaves, which have been shown to be temperature dependent. Six rice genotypes were planted at eight planting dates staggered at 15-day intervals in a rice garden in Lumle, Nepal. The appearance and development of successive leaves were observed up to leaf number twelve. Germination was linearly correlated and leaf appearance rate (LAR) was quadratically correlated with the mean air temperature. However, for all genotypes the influence of temperature on the LAR was less pronounced with each succeeding leaf appearance and ceased entirely after the appearance of leaf number five. For later leaves and particularly at later planting dates LAR decreased independent of temperature and leaf development rate slowed down. With limited resources plants need to balance sinks against sources. The development stage of leaf was expressed as a normalized senescence level or source status. These were added-up for all existing leaves on the main tiller. The resulting figure was used as an overall senescence indicator for the tiller. After leaf number eight, leaves were initiated at a genotype specific level of tiller senescence. The higher the senescence level the slower the leaf turnover in the canopy. For early planting dates genotypes having a low optimum temperature for leaf appearance need to be selected, whereas for later planting dates where early leaf development is fast due to higher temperature, leaf longevity becomes an important factor for a sustainable source for grain filling.

P43: Do apoplastic barrier bypasses exist in roots?

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Casparian bands function as a barrier to the movement of solutes within the apoplast of plant roots. This barrier has been determined by observing accumulation of fluorescent dyes and heavy metal tracers on the cortical side of the endodermis. However, Casparian bands develop at some distance from the root tip and can be interrupted by developing lateral roots where apoplastic leakages have been reported in earlier studies. Here we demonstrate Casparian band function in a model system using two mutants of *Arabidopsis thaliana* (*short-root* (*shr*) and *scarecrow* (*scr*)). Plants were grown for 12 days on vertical agar plates. On the twelfth day, the apoplastic tracer PTS (3-hydroxypyrene-5,8,10-trisulfonate) was applied and roots were allowed to transpire for 24h. Following incubation, leaves were harvested and PTS was extracted in 80% methanol. Leaf tissue extracts were analysed for PTS content using a Perkin Elmer microplate reader with 405nm excitation and 510nm emission filters.

PTS was observed in the leaves when Casparian bands were not present in the root (*shr* mutants) but not when the bands were present (*scr* mutants). These results are in agreement with published literature on Casparian band function. We have also observed the exclusion of PTS from the shoot system of wild-type *Arabidopsis*. Development of lateral roots in wild-type did not induce apoplastic leakages. The mechanisms that prevent the entry of PTS into the stele where Casparian bands are not yet developed at the root tip or have been interrupted by lateral root formation are unknown. Further research will involve determining PTS localization at these sites as an initial step in elucidating the mechanisms that prevent bypass around the apoplastic barrier formed by Casparian bands.

P44: The C-terminal tail sequences of ER and mitochondrial cytochrome b_5 convey their organelle-specific targeting in plant cells – conservation of the targeting mechanism for mitochondrial tail-anchored proteins

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Tail-anchored (TA) proteins are a unique class of membrane proteins that are targeted and integrated post-translationally into their respective organelles via unique targeting signals usually located at their C termini. One of the best studied TA proteins is cytochrome b_5 (Cb_5). Previously, we characterized the molecular targeting signals for both the ER- and mitochondrial-localized isoforms of Cb_5 by showing that the distinct physicochemical properties of their transmembrane domain and C-terminal tail sequence (CTS) dictate their organelle-specific targeting in plant cells *in vivo* (Hwang *et al.* 2004. Plant Cell 16:3002). Here we demonstrate that the CTS of ER-localized Cb_5 also conveys organelle targeting specificity by preventing spontaneous insertion into incorrect subcellular membranes. We show that while removal of the CTS of ER Cb_5 does not disrupt its targeting *in vitro*, it does result in a loss of targeting selectivity since the truncated protein displayed aberrant binding to mitochondria. The CTS of ER Cb_5 also was sufficient in conveying targeting specificity to another protein since replacement of the corresponding sequence in Bcl-2, a TA protein that is normally targeted to both ER and mitochondria, inhibited its mitochondria localization. Removal of the CTS from mitochondrial-localized Cb_5 also resulted in the truncated protein being mislocalized within the cell. These and other data including *in vitro* import experiments with proteolyzed ER or mitochondrial membranes suggest that the CTS-dependent targeting of Cb_5 involves specific proteineous factor(s) (e.g., receptors). Notably, the putative protein targeting/insertion machinery for at least mitochondrial-localized Cb_5 appears to be utilized also by almost all other mitochondrial TA proteins (e.g., Fis1, VAMP, mAPX, Tom20-1), since each of these proteins share the same C-terminal physicochemical properties as those described for Cb_5. Planned experiments aimed at testing this working hypothesis will be presented.

P45: The distribution of two major iridoids in different organs of *Antirrhinum majus* L. at selected stages of development

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Two iridoid glycosides isolated from leaves of *Antirrhinum majus* L. were identified as the known compounds antirrhinoside and antirrhide. These compounds were then quantified in different organs of plants grown hydroponically over the course of their development. Antirrhinoside was present in all plant organs including the roots at all developmental stages whereas antirrhide was found only in the leaves.

Furthermore, both iridoids were identified in the main stem axillary leaves and the leaves on the lateral branches. The highest concentrations of antirrhinoside were found in the main and lateral stems as well as the buds and flowers. As leaves age for both cultivars the levels of antirrhinoside drop significantly and there is a corresponding increase in antirrhide. In spite of the different genetic backgrounds of the two cultivars studied, the overall distribution of the iridoids was similar during vegetative and flowering development. Radiolabelling of recently expanded axillary leaves with $^{14}CO_2$ showed that both antirrhinoside and antirrhide were prominently labelled in the laminar tissue. However, only ^{14}C-antirrhinoside was recovered in the subtending petiole tissue, consistent with the suggestion that it is a phloem mobile compound.

P46: Two Arabidopsis plant U-box (AtPUB) E3 ubiquitin ligases may function as potential regulators of plant signalling pathways during abiotic stress

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The ability of plants to sense and respond to environmental and endogenous signals is essential to their growth and development. Ubiquitin-mediated proteolysis has emerged as an important process involved in how plant signalling pathways can be regulated in response to environmental or developmental cues. In plants, the involvement of the Ub-26S system during self-incompatibility was established by the characterization of the *Brassica* ARC1 protein, an E3 ubiquitin ligase that targets substrates presumed to be needed for compatible pollinations for degradation. In self-compatible *Arabidopsis thaliana*, there exists a 17-member family with strong homology to ARC1. Although the biological functions of these proteins have yet to be elucidated, based on their conservation of E3 ligase activity, these UND-containing AtPUB-ARMs appear to function in a manner analogous to ARC1. Interestingly, phylogenies based on domain sequence similarities have shown that two members, AtPUB18 and AtPUB19, consistently form a clade. General analysis of single T-DNA insertional lines for growth defects did not yield any distinct phenotypes, possibly due to the strong probability that both proteins operate redundantly. To alleviate the difficulty of functionally characterizing redundant proteins, expression profiling from publicly available microarray projects was performed. Intriguingly, for cold, osmotic, salt, and drought stresses, AtPUB19 mRNA levels were strongly up-regulated, while AtPUB18 mRNA upregulation was seen in osmotic and salt treatments. As a consequence of these analyses, condition-directed assays are being performed to determine *AtPUB18* and *AtPUB19* gene function and to assess whether AtPUB18 and AtPUB19 may in fact be involved in abiotic stress signalling.

P47: Dark septate endophytes: isolation, identification and characterization toward their potential use as biological control agents against plant pathogens

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Dark septate endophytes (DSEs) are fungi that colonize plant roots without producing disease symptoms or forming typical mycorrhizal structures. Their structural characteristics consist of darkly pigmented and septate hyphae which extend between and form microsclerotia within host cells. Despite their wide distribution in many plant species and in different ecosystems, their functions are still not well understood. Recently, studies have been conducted to investigate the potential of DSEs to act as biological control agents (BCAs) against foliar and soil-borne plant diseases. Nagano Prefecture is a major producer of field-grown lettuce in Japan and since 1995 there have been substantial crop losses due to root-rot caused by the soil-borne fungal pathogen, *Fusarium oxysporum* f.sp. *lactucae.* The objective of this

work is to isolate and characterize novel DSEs that may help protect lettuce against this and other plant pathogens by inducing the plants natural defense response, - Induced Systemic Resistance (ISR). DSEs were isolated from the roots of mature, healthy lettuce plants from 10 fields in the region. One morphotype of fungi with darkly pigmented hyphae was predominantly isolated and was designated "Type-a". Inoculation tests on lettuce performed under laboratory conditions confirmed that all of the isolates are capable of colonizing lettuce roots in a manner typical of DSEs including the formation of microsclerotia. Phylogenetic analysis of 3 isolates was performed by cloning and sequencing the genes for the small subunit (SSU) and large subunit (LSU) ribosomal RNAs (rDNA). rDNA sequences were aligned with 67 (SSU) and 69 (LSU) taxa including representatives from 10 or 8 classes within the Ascomycota, respectively. Both analyses suggest Type-a DSEs are members of the Sordariomycetes most closely related to but distinct from the Lulworthiales. Challenge experiments using *Ewinia carotovora* as the invading pathogen provide some indication that systemic resistance mechanisms may be induced.

P48: Growth temperature regulates colony formation in the psychrophilic and mesophilic strains of *Chlamydomonas raudensis*

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Chlamydomonas raudensis UWO 241 is a photosynthetic green alga isolated from the permanently ice-covered Lake Bonney in Antarctica. This unique psychrophile is adapted to an extremely stable environment of low temperatures and low irradiance. Recently, *C. raudensis* UWO 241 was identified as a strain of *Chlamydomonas raudensis* SAG 49.72, a mesophile from Meadow Lake in Czech Republic. As a consequence of adaptation to these distinct environments, UWO 241 and SAG 49.72 exhibit optimal growth temperatures at 8ºC and 28ºC, respectively. Morphologically, both of these strains have shown to exist as non-motile, multi-celled, membrane-bound "colonies" as well as motile, single cells. Quantitative light microscopy revealed that growth temperature has a profound effect on the development of "colonies" from single cells. Particularly, the proportion of "colonies" to single cells increased the psychrophile was shifted from low to high temperature. In contrast, this proportion increased when the mesophile was shifted from high to low growth temperature. The highest ratio of "colonies" to single cells was observed at the suboptimal growth temperatures of 11ºC in the mesophilic strain (SAG 49.72) and 16ºC in the Antarctic psychrophilic strain (UWO 241). In contrast, optimal growth temperatures of either 28ºC or 11ºC produced nearly homogeneous cultures of single cells in the mesophile and psychrophile, respectively. These results were confirmed by flow cytometry which indicated the presence of two distinct cell populations based on particle/cell size among SAG 49.72 cells grown at 11ºC and 28ºC. Further studies will examine the potential effects of cell morphology on the photosynthetic characteristics of single cells versus "colonies".

P49: Gene flow from *Sorghum bicolor* to its weedy relatives and its consequences

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Gene flow from the crop-to-wild direction is currently an issue of concern due to the upsurge in the introduction and commercialization of transgenic crops. Transgenes may get introgressed into weedy populations culminating in enhancing weediness. *Sorghum bicolor* has been shown to hybridize to *S. halepense* at a rate of 0-100%. Intraspecific gene flow has been detected up to 158 m. The current study was designed to test the hypothesis that; gene flow from *S. bicolor* to its wild relatives *S. halepense* and *S. sudanense* occurs at varying extents and it enhances the competitive ability of the hybrids. The crop and the two weedy relatives were characterized morphologically and for 55 simple sequence repeat (SSR) loci distributed across the 10 chromosomes of sorghum. The

morphological assays done included assays of: panicle shape, seed colour, culm length, seed set and days to half bloom. The analyses showed that the three species are divergent with *S. halepense* being furthest from both *S. bicolor* and *S. sudanense*. Polymorphisms were obtained on at least one marker on each of the ten chromosomes (A-J) with chromosome 10 showing polymorphism for three markers. Interspecific hybridization occurred in the *S. sudanense x S. bicolor* and *S. halepense x S. bicolor* handcrosses at frequencies of 40% and 25%, respectively. The polymorphic SSR markers identified above will allow estimation of the extent and pattern of hybridization in the progeny of the crosses. Competiveness of the weed/crop hybrids will be measured in a replacement series planting with the parents to determine relative germination, tillering, plant growth, time to flowering, grain weight and leaf weight.

P50: Effects of cadmium on stomatal size and oxygen evolution

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Scanning electrochemical microscopy (SECM) has been used for the first time to investigate the effects of cadmium-induced stress on *Brassica juncea* (L.). The SECM probe detects changes in electrical current arising from changes in topography of the sample as well as electrochemical changes at the tip of the probe. The resolution of the SECM probe fabricated in Ding's lab was sufficient to produce clear images of stomata. Compared to control plants, those treated with 0.2 mM $CdCl_2$ had lower stomatal density and larger stomatal width. This suggests that cadmium inhibits the differentiation of guard cells in the plane of the epidermis and/or fewer stomata were open in cadmium-treated plants. To monitor oxygen evolution, the probe was placed above individual, fully open stomata (as determined by swollen guard cells). *In vivo* oxygen evolution per stoma in cadmium-treated plants was less than for control plants, as determined from the current above the centres of the stomata. Thus, decreased oxygen evolution in response to cadmium cannot be solely attributed to reduced stomatal density or simple closing of the stomata; cadmium also induces a lower photosynthetic yield. Conventional measurement of photosynthetic activity also permits rapid *in vivo* assessment, but only for an average response over an area $\geq$ 1 cm^2. Our results illustrate that SECM provides an excellent tool for monitoring *in vivo* the effects of stress on a photosynthetic activity at the scale of individual stomata.

P51: **Phosphorylation of PS II core proteins in *chlorina F2* barley mutant (*Hordeum vulgare* L. cv .Dornaria, Chlorinaf2-^{2800})**

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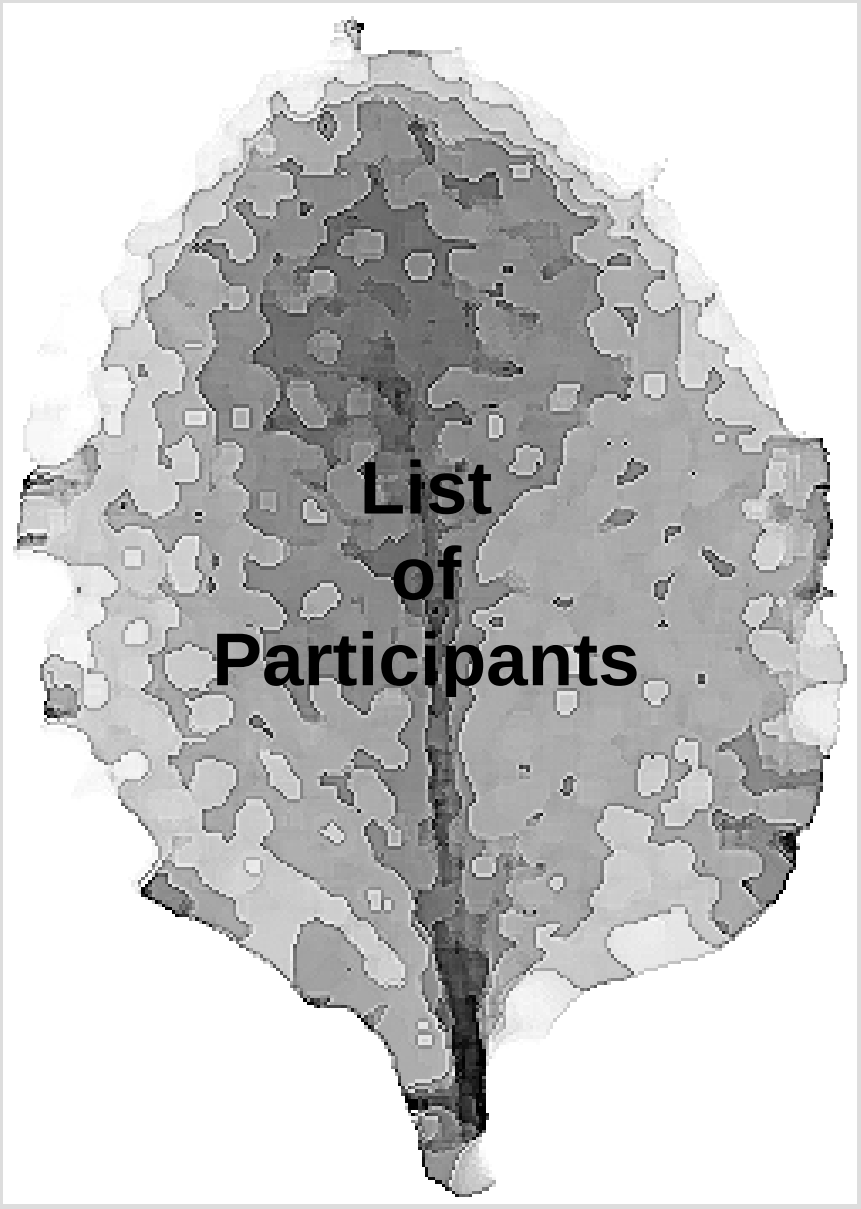
Chlorina F2 barley mutant is a single nuclear gene mutant, controlling the accumulation of Chl.*b* (Leverentz et al., 1992). This mutant lacks the Lhcb1 polypeptide, has a reduced amount of Lhcb2-4, while exhibiting no affect on Lhcb5. In the present study we demonstrate that the absence of Chl *b* in the *Chlorina F2* mutant has an effect on D1 protein phosphorylation, degradation, and stability after protease treatment. Phosphorylation of Lhcb is the major mechanism controlling the distribution of excitation energy between PSII and PSI, in a process known as state transition. Reduction of plastoquinone (PQ) and binding of plastoquinol (PQH) to the cytochrome b_6/f complex is required for Lhcb phosphorylation (Bennett et al.,1988). According to Forsberg and Allen (2001), phosphorylation of D1/D2 and Lhcb proteins are under the control threonine/serine/tyrosine kinases superfamily. Threonine, serine and tyrosine residues of thylakoid proteins are typical targets of these kinases. Using phosphothreonine antibody we were unable to detect D1 protein phosphorylation in the *F2* mutant. However, we were able to detect it using ^{32}P under *in vitro* and *in vivo* phosphorylation condition. This discrepancy has caused us to search for which of the three amino acids (threonine, serine, and tyrosine) are phosphorylated on the D1 polypeptide in the *F2* barley mutant.

Notes

Notes



List of Participants

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Fadi	Al-daoud	McMaster University	P 13
Wendy	Allan	University of Guelph	O V1.3
Chuthamat	Atnaseo	University of Guelph	P 20
Patrick	Audet	University of Ottawa	O V1.4
Bahman	Bahramnejad	University of Guelph	P 19
Joyce	Baxter	University of Toronto	O V.7
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Clifford	Beninger	University of Guelph	P 45
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Michael	Emes	University of Guelph	P 22, P37
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Scott	Fife	University of Guelph	P 8
Ashok	Ghosh	Brock University	
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Xuemei	Gong	University of Guelph	O II.1, O IV.1
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